# Risk and Impact of Insect Herbivores on the Development of Dryland *Eucalyptus* Forestry in New Zealand

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# ABSTRACT

*Eucalyptus* plantations in New Zealand are occupied by a number of exotic insect defoliators and have increasing risks of new pest incursions. Pest outbreaks causing significant defoliation can reduce tree growth and productivity. Integrated pest management (IPM) strategy is useful to reduce potential risk of insect outbreaks and minimise pesticide use that has negative impacts on the environment. However, IPM in forestry plantations in New Zealand is still in its infancy. An industry centred on the production of naturally durable wood products is being developed in dryland areas in New Zealand. One of the priority species in the emergent industry is *Eucalyptus bosistoana,* which is drought tolerant and can produce highly durable heartwood. For durable species, including *E. bosistoana* to be considered as a commercially valuable option for planting in the future, we need to understand the risk and impact of currently present insect defoliators on these species.

Understanding the population dynamics of key insect defoliators is essential to predict their outbreak potential. Hence, insect surveys were conducted for *Paropsis charybdis*, *Opodiphthera eucalypti, Strepsicrates macropetana* and *Phylacteophaga froggatti* over two growing seasons in a dryland *E. bosistoana* site. Additionally, an insect development assay was conducted in the laboratory to attain base temperatures and degree-day requirements (DD) of life stages of *P. charybdis* (the most important eucalypt insect pest in New Zealand) to construct a DD model to simulate its phenology. Results showed that the observation of one generation of *P. charybdis* was different from previous studies, likely due to the drought conditions at the site. One to two generations were observed for *O. eucalypti*, and multiple overlapping generations were observed for *S. macropetana* and *Ph. froggatti*. The model was most capable of predicting voltinism of *P. charybdis* under the scenario assuming longer DD requirement of median egg laying age and hibernation start by 20 March, or a scenario with assumptions of shorter DD requirement of median egg laying age, hibernation start by 20 March and later over-winter adult emergence date (late September). Prediction of appearance of life stages was not highly accurate, but models that assumed shorter DD requirement for the median egg laying age tended to be the most accurate.

To assess the impact of defoliation on the growth of young *E. bosistoana* in dryland area, a trial simulating different defoliation severity (moderate and severe defoliation) and timing (spring and late summer, and spring plus late summer) effects on the growth of *E. bosistoana* was conducted. Only spring moderate defoliation did not significantly reduce tree growth, while other defoliation treatments significantly reduced either diameter or height growth. Severity of defoliation had a negative relationship with tree growth, but there was no significant difference observed between moderate and severe defoliation treatments. Late summer defoliation had a larger impact than spring defoliation, and this was exacerbated by defoliation severity. Repeated defoliation had greater negative impact on tree growth relative to single defoliation events. These results imply that spring moderate defoliation may not require pest control.

With the objective to identify families of *E. bosistoana* that have higher/lower resistance or tolerance to insect defoliation, and the most suitable method for this purpose, tree health assessments were conducted on 14 *E. bosistoana* families and 1 *E. globoidea* family using four assessment methods (based on defoliation levels and pest loads) over two growing seasons in

an *E. bosistoana* site. Significant variation in insect susceptibility and tolerance was found between *E. bosistoana* families to the examined pest species except *O. eucalypti*. Southern provenance families were found to be more insect tolerant. The single *E. globoidea* family and Family 125 (Bungonia provanence) of *E. bosistoana* were found to be relatively fast growing and resistant to examined pests. These families should be maintained in the breeding programme.

To assess the between species variation in susceptibility to *Paropsisterna variicollis*, tree heath assessments were also conducted on 11 durable eucalypt species at three dryland sites in the Hawke's Bay region in the North Island. Significant between species variation in defoliation and pest loads of *Pst. variicollis* was observed. The most susceptible species tested were *E. tricarpa* and *E. bosistoana*, while the least susceptible species were *E. macrorhyncha*.

Implications of the thesis cover three aspects of IPM, including pest monitoring, defining control action thresholds and tree improvement (selective breeding) to reduce insect outbreaks. The findings from this thesis can be applied more broadly to the sustainable IPM of the developing New Zealand durable eucalypt industry and the wider plantation forestry industry.

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# CHAPTER 1 INTRODUCTION

## 1.1. Dryland *Eucalyptus*

### 1.1.1. Background

*Eucalyptus* is one of the most planted hardwoods in the world (Potts et al. 2011), with 50% of all plantations in the tropics consisting of five *Eucalyptus* species (Sunder 1993). The world area of *Eucalyptus* forests is more than 19 million ha, mainly in Brazil, China and India (Iglesias-Trabado and Wilstermann 2008). *Eucalyptus* is primarily grown to produce pulp and paper, however some species can provide construction timber. It is also used in cleaning and insect repellent products and for bioenergy production. Besides their economic value, plantation *Eucalyptus* also delivers ecological values. Plantings of eucalypts contribute to the protection of land and water systems, such as erosion control and flood mitigation, and it is one of the best genera used as a carbon sink due to its efficient biomass production (Branco et al. 2015). *Eucalyptus* can also provide floral resources for commercial honey bees, and habitats for native birds and insects.

In New Zealand, the most widely grown plantation species, *Pinus radiata* D. Don, takes up 1.5 million ha (90%) of the forest plantation area, while *Eucalyptus* species only occupy 23,000 ha and account for 1% of timber production (FOA 2012/2013). However, over-reliance on a single speices limits available markets and, importantly, poses a high biosecurity risk to the forestry industry (Apiolaza et al. 2011a). Arrival of a major pest or pathogen can cause considerable damage to trees and disrupt trade (Brockerhoff and Bulman 2014, Pawson et al. 2014). Moreover, *P. radiata* has a number of short-comings including environmental contamination and trading issues. Timber from *P. radiata* does not last for a long time in contact with the ground without preservative treatmentment. The majority of poles for vineyards in New Zealand and Australia is made from *P. radiata* treated with the preservative copper chrome arsenic (CCA), which poses an environmental problem (Bush and Walker 2011). Specifically, in the South Island of New Zealand, over 500,000 CCA preserved *P. radiata* poles are supplied to vineyards annually (NZDFI 2013). These preserved poles require replacement about every 2 decades and are required to be disposed of in secure landfills due to the potential for leaching of heavy metals into groundwater (Walker 2013). Moreover, CCA has been banned for many uses by America, Australia and some European countries (Nicholas and Millen 2012), limiting market access for New Zealand *P. radiata* products.

In contrast to *P. radiata*, some *Eucalyptus* species produce naturally ground durable heartwood, which can resist biodeterioration caused by bacteria, fungi, termites, borers and marine organisms in outdoor conditions without chemical treatments. This is due to the presence of wood extractives, which can act as fungicide and antioxidants (Schultz and Nicholas 2000, Bush and Walker 2011). Although New Zealand has been limited in developing large estates of *Eucalyptus* plantation for pulp and energy production and is unable to compete with other pulp producing countries like Brazil and Uruguay, market opportunities for naturally durable eucalypt give New Zealand a new opportunity to breed elite eucalypt species. The potential demand for naturally durable *Eucalyptus* wood products far exceeds the current yield (Nicholas and Millen 2012). Other durable hardwoods exist, but sustainable forest practices in tropical

countries where the hardwoods are grown can be uncertain. Domestic New Zealand and international markets have recently expressed more interests in naturally ground durable *Eucalyptus* timber that does not require chemical preservative to maintain wood integrity (Maclaren 2005, Nicholas and Garner 2007).

Besides the increasing demand for naturally durable timber, tolerance of *Eucalyptus* to drought, ability to thrive in soil of low nutrient availability and adaptibility to eroding landscapes (Smethurst and Walker 2011), make them good candidates for planting in dryland areas. New Zealand dryland areas, which receive rainfall of 500 to 100 mm annually, and occupy 19% of New Zealand land area, are not suitable for planting *P. radiata* (Apiolaza et al. 2011b, Norbury et al. 2015), but *Eucalyptus* plantations are being considered as alternatives to agriculture and sheep farming in these areas with the potential to provide greater financial returns and also environmental benefits (Smethurst and Walker 2011). In their native range, *Eucalyptus* have adapted to a wide diversity of habitats from humid to semi-arid areas, from sea level to alpine, and from the tropics to temperate zones (Potts et al. 2011). Therefore, it is possible to find some species that are suited to New Zealand's drylands. Moreover, due to more rapid growth rates and higher density timber compared with *P. radiata*, durable eucalpyts are highly eligible for New Zealand's Emissions Trading Scheme (Apiolaza et al. 2011a). Walker (2013), Apiolaza et al. (2011a) and Apiolaza et al. (2011b) have domentrated these opportunities for planting drought torlerant *Eucalyptus* species for naturally ground durable timber products in dryland areas in both the North and South Island.

### 1.1.2. The NZDFI programme and *Eucalyptus bosistoana*

The New Zealand Dryland Forests Initiative (NZDFI) is a sustainable commercially-oriented research project established in 2008. The project aims to breed and improve drought tolerant *Eucalyptus* species that can produce ground-durable timber that does not require chemical treatment (Van Ballekom and Millen 2017). Natural durability is rated using a four-class system based on the probable life expectancy of heartwood under in-ground, above-ground and marine exposure under Australian Standard 5604-2005 (Bush and Walker 2011). Among the primary species (*E. argophloia, E. bosistoana, E. globoidea, E. quadrangulata* and *E. tricarpa*) in the NZDFI breeding project for genetic improvement, *Eucalyptus bosistoana* F.Muell. (subgenus *Symphyomyrtus*) is the priority species due to its drought tolerance, high heartwood durability, ability to coppice vigorously after fire and harvesting, and because it does not appear to spread into native ecosystems (Nicholas and Millen 2012). It is naturally distributed along the south-eastern coast of New South Wales, Australia (between 33-37.5ºS latitude), from Sydney southwards to the eastern Gippsland area of Victoria (Brooker and Kleinig 2006), between sea level and 500 m (Nicholas and Millen 2012). It is a medium-sized to tall tree averaging 30-40 m in height in Australia, with variable morphology. Juvenile leaves are petiolate, ovate to orbicular and adult leaves are petiolate, lanceolate or narrow-lanceolate (Brooker and Kleinig 2006). Wood from *E. bosistoana* is used for construction, poles and fences (Bootle 1983). Experience in planting *E. bosistoana* is limited, including knowledge about its silvicultural and pest management requirements. Damage by two common pests in traditional eucalypt plantations in New Zealand, *Paropsis charybdis* Stal (the *Eucalyptus* tortoise beetle) and *Acrocercops laciniella* (Meyrick) (the blackbutt leafminer) *on E. bosistoana* has been recorded in Northland (Nicholas and Millen 2012) and some NZDFI Marlborough trials (Murray unpublished data). Several other insect pests have also been

observed feeding on *E. bosistoana* in some NZDFI trials, but its susceptibility to insect damage and ability to recover from defoliation remains unknown. For this species to be considered as a commercially valuable option for planting in the future, we need to know the risk and impact of insect pests on its growth.

## 1.2. Risk of insect pests

### 1.2.1. Pests from Australia

Historically, New Zealand has had limited success in establishing large estates of *Eucalyptus* plantations. This is partly because of limited collection of genetic material for breeding to explore the between- and within- species variation in growth in the local environment. However, pests and diseases have also played a significant role in reducing the success of New Zealand's eucalypt industry (Apiolaza et al. 2011a). Forestors in New Zealand admit that a key factor in historic failures to establish eucalpyt plantations was insect pest attack, especially by the leaf-feeding beetle *Paropsis charybdis* Stal (Clark 1930, Apiolaza et al. 2011a).

According to invasion ecology theory, exotic *Eucalyptus* plantations in New Zealand (and elsewhere) are at great risk of attack from exotic insect pests (Withers 2001). Because eucalypts are rarely fed by native insects, when grown as monocultures, they provide a vacant niche for Australian eucalypt pests that arrive, and these pests can take advantage of the resource in the absence of natural enemies (Withers 2001). Due to the short distance and frequent travel and trading activities between New Zealand and Australia, many Australian eucalypt-feeding insects have established in New Zealand, arriving by aerial dispersal, tourism and commerce (Paine et al. 2011). Eucalypt pests of Australian origin have colonized New Zealand's eucalypt plantations since the 1860s (Withers 2001). Rates of colonization peaked in the 1990s at around one insect every 18-months (Withers 2001), but have dropped in recent decades to around one insect every five years (Withers 2001, Withers and Bain 2009). To date, at least 34 eucalypt specialists have established in New Zealand, primarily Coleoptera, Lepidoptera and Hymenoptera, and sap-sucking psyllids (Murray and Lin 2017). Most recently, in 2016, the *Eucalyptus* variegated beetle (EVB), *Paropsisterna variicollis* (Chapuis), was detected for the first time, and has been confirmed to be widely established in the Hawke's Bay region (Rogan 2016).

### 1.2.2. Key insect defoliators in the South Island dryland areas

Although most damage from the established *Eucalyptus* feeders in New Zealand is minor to moderate, occasional severe local outbreaks occur, around one third of which require control (Withers 2001). In temperate regions, 75% of key plantation pests are defoliators (Eyels et al. 2013), and most of these are insects. Several insect defoliators have been observed (by the author and others) feeding on plantation eucalypts in dryland areas in New Zealand, including, but not limit to, the gum leaf skeletoniser (*Uraba lugens* Walker (Arctiidae: Nolinae)), the bronze bug (*Thaumastocoris peregrinus* (Hemiptera: Thaumastocoridae)), the *Eucalyptus* tortoise beetle (*Paropsis charybdis* Stål (Coleoptera: Chrysomelidae)), the gum emperor moth (*Opodiphthera eucalypti* Scott (Lepidoptera: Saturniidae)), the *Eucalyptus* leafroller (*Strepsicrates macropetana* Meyrick (Lepidoptera: Tortricidae)) and the *Eucalyptus* leaf-blister sawfly (*Phylacteophaga froggatti* Riek (Hymenoptera: Pergidae)). Some *Eucalyptus* insect

pests in New Zealand are insignificant or even unknown in their native ranges in Australia. As such, information on their damage to *Eucalyptus* growth and their phenology is scarce, or even absent before they arrived in New Zealand. The phenology of some of these pests has been assessed in the North Island of New Zealand, but is relatively unknown in the South Island context, where climatic conditions are different.

In this section I will review the phenology, biology and pest potential of the four major insect herbivores (Table 1.1) that are the focus of this thesis. These species, *P. charybdis*, *O.eucalypti, S. macropetana* and *Ph. froggatti,* have all been noticed damaging *E. bosistoana* in the South Island**.**

**Table 1.1** Brief descriptions of the focused insect herbivores.

| Species | Common name | Damage type | Feeding preference | Main control agents present | Phenology studies |
|---|---|---|---|---|---|
| *Paropsis charybdis* (Coleoptera: Chrysomelidae) | *Eucalyptus* tortoise beetle | Scalloped chewing damage, "broom top" | Larvae - young foliage; adult- young and adult foliage | *Enoggera nassaui* (Girault), *Neopolycystus insectifurax* | Styles, 1970; McGregor, 1989; Murphy & Kay, 2000 (NZ North Island) |
| *Opodiphthera eucalypti* (Lepidoptera: Saturniidae) | Gum emperor moth | Chewing damage, similar to *P. charybdis* | Larvae feed on all kinds of foliage | A polyhedral virus; a parasitic fungus *Beauveria sp.*, magpies | |
| *Strepsicrates macropetana* (Lepidoptera: Tortricidae) | *Eucalyptus* leafroller | Brown skeletonised surface, leave at shoot tips woven together | Larvae feed on leaves, buds and developing flowers, especially on young trees | *Trigonospila brevifacies* | Mauchline *et al*., 1999 (NZ North Island) |
| *Phylacteophaga froggatti* (Hymenoptera: Pergidae) | *Eucalyptus* leaf-blister sawfly | Brown blister on leaf upper surface | Upper leaf surface of maturing foliage | *Bracon phylacteophagus; rain* | Farrel & New, 1980; Loch, 2004 (Australia) |

***Paropsis charybdis* (the *Eucalyptus* tortoise beetle)**

*Paropsis charybdis* was first recorded from the Port Hills in Canterbury in 1916 (Styles 1970), and was recognized as a significant pest of eucalypt plantations as early as the 1930s (Clark 1930; Styles 1970). In 1928, Clark (1930) found *P. charybdis* had infested the eastern part of the South Island, south to Dunedin and north to the Kaikoura Ranges. It was found in Nelson in 1932 and extended to Southland by 1938. Establishment in the North Island was not

confirmed until the late 1950s, but soon after that in 1956, *P. charybdis* had colonized the whole country (White 1973).

No native natural enemies provide good control of *P. charybdis* in New Zealand (Clark 1930). Historically, chemical control only lasts for a short time and aerial spraying is too expensive due to the relatively small size of eucalypt plantations in New Zealand (Styles 1970). Today, the requirement for environmental sustainability requires chemical control to be minimized. Considering these factors, biological control is regarded the most appropriate way to control *P. charybdis*. The egg parasitoid *Enoggera nassaui* Girault was successfully introduced in 1987 and a second biotype was imported in 2000 (Mansfield et al. 2011). However, the self-introduced hyperparasitoid *Baeoanusia albifunicle* Girault is thought to have reduced the efficiency of *E. nassaui* as a biological control agent for *P. charybdis* (Jones and Withers 2003, Murray 2010, Mansfield et al. 2011). Another egg parasitoid, the self-introduced *Neopolycystus insectifurax* (Girault), appears to be immune to *B. albifunicle* and may partially compensate for the decline in *E. nassaui* (Mansfield et al. 2011). To improve the control of *P. charybdis*, a larval parasitoid, *Eadya paropsidis* Huddleston and Short (Hymenoptera: Braconidae: Euphorinae), is being assessed (Withers et al. 2017).

The lifecycle and behaviour of *P. charybdis* have been extensively studied in the North Island (Styles 1970, McGregor 1989, Murphy and Kay 2000) where it has two generations each year. Larval feeding by the first generation is the most damaging, but pre-winter feeding by second-generation adults causes significant damage at the end of summer, potentially preventing re-foliation before winter. McGregor (1989) investigated the life-cycle, behaviour and development of *P. charybdis*, and conducted a very detailed review of biology studies on *P. charybdis*.

***Opodiphthera eucalypti* (the gum emperor moth)**

The first record of *O. eucalypti* in New Zealand was in Wanganui in 1915 (Crosby et al. 1976). Although its natural rate of spread is slow, transportation by man has helped the species establish in both the North and South Island (White 1972). The life cycle and phenology of gum emperor moth has been presented in White (1972), Alma (1977) and Phillips (1993), but without detailed descriptions on how the data was obtained. Adults emerge from October to mid-December or even February depending on local climate. Larvae can be found from November to February. Two generations can be seen in the field annually (White, 1972). Larvae have been reported to feed on many tree species including 18 *Eucalyptus* species and some introduced deciduous trees (White 1972, Meyer-Rochow 1986). The number of *Eucalyptus* hosts may have increased in New Zealand as more eucalypt species have been established. *Eucalyptus bosistoana* and other NZDFI species have not been assessed as hosts for *O. eucalypti,* but it has been observed feeding on *E. bosistoana.*

***Strepsicrates macropetana* (the *Eucalyptus* leafroller)**

The *Eucalyptus* leafroller was first recorded in New Zealand in 1923 in Auckland (Crosby et al. 1976) and develops on a wide range of *Eucalyptus* species, including *E. nitens*, *E. saligna*, *E. fastigata* and *E. regnans* (Mauchline et al. 1999, Mauchline et al. 2001). Although there is an intentionally introduced biological control agent, *Trigonospila brevifacies* Hardy (Diptera:

Tachinidae), *S. macropetana* is now widely distributed throughout New Zealand. The insect feeds primarily on juvenile foliage but also shoot tips, buds and developing flowers, so young trees can be severely damaged.

The life history of *S. macropetana* has been studied in the laboratory (Mauchline et al. 1999). There are five larval instars and overall development takes about 46 days under laboratory conditions. The insect was predicted to have at least two or perhaps between six and eight generations annually based on the development time and temperature. A field survey in five sites within the Manawatu and eastern Bay of Plenty found that some *Eucalyptus* species, including *E. globoidea* and *E. regnans*, were less susceptible to feeding damage by *S. macropetana* (Mauchline et al. 1999).

***Phylacteophaga froggatti* (*Eucalyptus* leaf-blister sawfly)**

The *Eucalyptus* leaf-blister sawfly is native to south-eastern Australia and has spread to south-western Australia, Tasmania, New Zealand and New Caledonia (Loch et al. 2004). The *Eucalyptus* leaf-blister sawfly was first found in New Zealand in 1985, near Auckland Airport (Crosby et al., 1976). Although the current economic impact is limited in its native range in south-eastern Australia, it can be a common and serious pest in its invaded locations (Stone and Birk 2001, Loch et al. 2004, Nahrung et al. 2012). The pest has spread throughout the North Island, and as far south as Dunedin in the South Island and feeds on a wide range of *Eucalyptus* species. The pest has also been found feeding on other species, such as *Quercus* (oak) and *Betula* (birch) (Kay 1986). Larvae of *Ph. froggatti* damage leaves by mining in the upper leaf surface of maturing foliage, feeding on mesophyll tissue and leaving a brown "blistered" appearance on the leaf (Farrell and New 1980, Kay 1986). There are no native natural enemies of *Ph. froggatti* in New Zealand, but the parasitoid, *Bracon phylacteophagus* Austin (Hym.: Braconidae) was introduced to New Zealand in 1988. The parasitoid has spread through the infested area and successfully controlled the population of *Ph. froggatti* in the North Island (Faulds 1990, 1991).

Life history and phenology studies of *Ph. froggatti* have been conducted in Australia, but no study is found in the New Zealand context. Complete development has been shown to take 6 weeks in the laboratory and 40 days on 3-year-old *E. botryoides* in the Victoria, with overlapping generations in the field (Farell & New, 1980). Significant overlap in generations was also observed in south-western Australian blue gum plantations (Loch et al. 2004). These observations imply that there may be several generations of the pest in the field each year, and both studies indicated that most damage from *Ph. froggatti* in Australia occurs between autumn and spring. Local conditions can also influence the insect's voltinism (Farrell & New 1980).

Due to the changeable and different climate patterns, phenology studies in other regions, including the South Island areas are needed to gain a better understanding of the population dynamics of the above eucalypt defoliators.

## 1.3. Integrated pest management

### 1.3.1. Why manage pests in an integrated and sustainable way?

Sustainability is the future direction of forest management, since it is socially desirable and environmentally and economically beneficial in the long term. Forestry managers are increasingly encouraged to attain environmental certifications for their forest products (Elek & Wardlaw, 2013), such as certification from the Forest Stewardship Council (FSC). Mission and vision of FSC is to protect forests for future generations, and their principles require that plantations need to be managed such that they benefit not only the economy but also the environment, conservation and local community values, highlighting the need for monitoring and assessment of forest condition, activities, products and their social and environmental impacts (FSC 2015). One of the requirements of FSC is to reduce the input of herbicides and pesticides because of their negative environmental impacts. Integrated pest management (IPM) is one way of achieving this.

IPM combines multiple techniques that are effective and environmentally friendly, to prevent or reduce pest damage below an economic injury level on a long-term scale (Zanetti et al., 2014; UCIPM, 2014; EPA, 2014; Fettig et al., 2005). IPM programmes use comprehensive information on the biology and phenology of target pest species and their interactions with hosts and the environment to manage pest damage while minimizing possible harm to the environment and human health (EPA 2014). Therefore, insect-host interactions are the core area that needs to be understood to develop successful IPM programmes. IPM, particularly for agricultural crops, has made considerable progress in the past 30 years, but IPM in intensively managed plantation forests, including determining appropriate damage and population assessment methods, action thresholds and control methods, is not well developed for many pests (Coyle et al. 2005). Also, there are many countries still lacking experience and knowledge in establishing and operating IPM (Alao et al., 2011; Chungu et al., 2010).

IPM programmes vary but they consist of some common parts. These include prevention (e.g. selecting sites and resistant species), monitoring and assessment (e.g. identifying pest, population monitoring, damage assessment and setting action thresholds), silvicuture (e.g. fertilizing and modelling tree growth under different conditions) and control (e.g. chemical control, biological control and timing of insecticide application) (EPA, 2014; Elek and Wardlaw, 2013; Wardlaw 2011; Eyles et al., 2013; Eyles et al., 2010; Eyles et al., 2008; Waring & Hara, 2005). Elek and Wardlaw (2013) reviewed the options for managing chrysomelid leaf beetles in Australian *Eucalyptus* plantations, highlighting the trend towards sustainable and long-term "landscape" management that persists for the duration of rotation. They emphasise implementing control only when there is significant potential for an outbreak, and advocate for the importance of developing less susceptible plantation stock. Phenotypic expression of resistance to herbivore defoliation is influenced by the environment, but exactly how and the relative impacts of different environmental factors are largely unknown for *Eucalyptus* (Eyles et al., 2013). Furthermore, our understanding of induced resistance (IR) within *Eucalyptus* species is very poor, despite its potential application in sustainable forest management (Eyles et al., 2010). While IPM programmes for *Eucalyptus* plantations have been developed and put into practice in countries such as Australia (Candy 2000b) and America (Paine et al. 2000) in the last two decades, in New Zealand such practice is still in its infancy. Due to increasing numbers of pest species incursions in New Zealand and changing global climate conditions, more effort needs to be directed into IPM programme development to ensure the healthy and

sustainable management of *Eucalyptus* plantations. This should include the development of pest population monitoring methods and damage assessment tools, tree improvement (selecting resistant or tolerant stock), understanding induced resistance, defining economic damage thresholds and modelling the physiological response of host species to damage (Eyles *et al*., 2013).

### 1.3.2. The importance of pest monitoring

Evidence suggests that some insect pest attacks have become more intense in recent years. In the eastern USA, *Adelges tsugae* Annand (Hemiptera: Adelgidae) could cause 80%-90% mortality of the Eastern and Carolina hemlocks (Branco et al., 2015), while on the Colorado Plateau and Rocky Mountains, *Dendroctonus ponderosae* Hopkins (Coleoptera: Curculionidae), has killed 1 million ha of western yellow pine and 1.5 million ha of piñon pine (Boyd et al. 2013). In many cases, chronic and severe damage from insect defoliators was a major contributor to tree dieback (Ohmart and Edwards 1991).

Selecting eucalypt species that are well suited to the local climate and environmental conditions in New Zealand should minimise pest damage by ensuring plantations consist of healthy fast growing trees (Wardlaw 2011). However, even if good species-site matching is achieved, a pest monitoring programme is crucial for the long-term management of the industry due to the number of established pests, their occasional outbreaks, and the continuing risk of new incursions (Withers 2001, Murray and Lin 2017). Monitoring and assessment of pest populations and damage is an essential part of IPM, although developing suitable methodology is another practical problem. America (e.g. the USA forest health monitoring programme), Britain and Australia have regular surveillance and field guidelines for monitoring and damage assessment in hardwood forests and *Eucalyptus* plantations (Redfern and Boswell 2004, Stone and Coops 2004). However, only a limited number of potential pests has been evaluated for their phenology and damage. As a result, when severe damage occurs, forest managers often revert to using chemical insecticides (Coyle et al. 2005). Different insect damage assessment methods have been used, but no standard protocols have been developed and applied for large woody trees relative to the more accessible crops and low fruit trees in IPM programmes historically. For example, in Australia, various crown damage assessment methods were used to monitor the impact of chrysomelid beetles before the currently used Crown Damage Index (CDI) was developed, but application in mature trees is still challenging due to crown size and canopy closure (Stone *et al.,* 2003). As most *Eucalyptus* defoliators in New Zealand are either unknown or not significant pests in Australia, or elsewhere, there is little information on their population dynamics. Therefore, developing meaningful monitoring programmes is essential for understanding their phenology and damage levels and outbreak potential in New Zealand to help forest managers decide when and where to apply control. Further, a better understanding the phenology of pests can help facilitate more effective and resource efficient monitoring programmes.

### 1.3.3. Screening for suitable genotypes in tree breeding programmes

In forest plantation development, traits for defence against insect attack have been overlooked compared with those for productivity and wood quality in many breeding programmes, due to

limited time and also financial input. However, as the threats posed by insect pests have become increasingly severe in most plantation areas due to the growing numbers of unexpected new insect incursions and predicted insect responses to future predicted climate change (Wingfield et al., 2013; Henery et al., 2011), the importance of tree breeding to manage insect issues has gained more recognition. Pest resistance/tolerance traits are recommended for consideration in breeding programmes when known pests can severely impact on priority objectives (usually growth and wood property) (Potts et al., 2011). Boshier and Buggs (2015) reviewed studies on field trials and genomic technology to enhance resistance to pests in plantations. In the review, breeding sitka spruce (*Picea sitchensis* (Bong.) Carr.) resistant to white pine weevil (*Pissodes strobi* Peck) was identified as an example of a programme which specifically aimed to breed for pest resistant plantation species (King & Alfaro, 2009; Hall et al. , 2011). In another example, selecting insect-resistant clones of *E. grandis* x *E. urophylla* hybrids has increased planting stock and forest health in Brazil and South Africa (Wingfield et al., 2013). Insect resistance was also considered in a commercial breeding programme of willows started in 1987 (Larsson 1998).

The difficulties in selecting insect resistant *Eucalyptus* has been reviewed by Henery et al. (2011). In addition to the trade-off in time and resources that are put into selection for growth traits over pest defence, they noted selection leads to decreased genetic diversity which may increase susceptibility to outbreaks of other pests. They also noted the potential for host switching over time, which results in previously less preferred species becoming more susceptible. Nevertheless, new technologies, such as DNA-based tools for identification, detection and monitoring of pests, and progress in breeding and selecting less susceptible species and hybrids, have given *Eucalyptus* plantations an optimistic future (Wingfield et al., 2013). Moreover, while selecting for insect resistant stock has been more common historically, selecting for insect tolerant stock is another approach that could be considered to reach the same purpose. This is backed up by several plant defence theories that argue fast growing, healthy plants can tolerate higher levels of herbivory when grown in a suitable environment (Stone 2001). The ability of *Eucalyptus* to recover from insect damage (i.e. pest tolerance) has been investigated for a limited number of *Eucalyptus* species, with highly variable results depending on the species and growing locations. This content will be discussed in the following section.

### 1.3.4. Determining an acceptable defoliation threshold

If a threshold level of damage below which insect control is not necessary can be determined, unnecessary chemical input into the environment can be avoided and economic cost to growers can be reduced due to more efficient use of pesticide. Such economic thresholds have been used in the management of various forest plantations. For example, oil palm plantations in Malaysia (Darus and Basri 2000) and *Eucalyptus* forests in Tasmania, Australia (Elliott et al. 1992). The net benefit from a Chrysomelidae IPM programme which integrated a threshold level of defoliation for chemical control was over $400,000 (Wardlaw et al. 2010). Setting this threshold can improve the effectiveness of pesticide use, reducing the impact on the environment and is beneficial for natural enemies.

*Eucalyptus* is renowned for its ability to recover form fire and defoliation. This ability is attributed to the up-regulation of photosynthetic rates of the remaining leaves (Pinkard et al.

2011a). Studies on defoliation impacts on the growth of a few widely planted *Eucalyptus* species concentrate on the last two decades in Australia. These include stuidies on insects (e.g. Quentin et al. 2010), myco-sphaerella leaf disease (MLD) (e.g. Lundquist and Purnell, 1987), and mammals (Bulinski & McArthur, 1999). The responses of eucalypts to defoliation by these different agents have been found to differ, for example, *Eucalyptus* was found to have higher tolerance to insect damage compared to MLD in general (Carnegie and Ades 2003). However, these studies have only been conducted on a very limited number of commercially planted *Eucalyptus* species, such as, *E. globulus*, *E. nitens* and *E. regnans*. Although these species can generally tolerate moderate levels of defoliation caused by insects, highly variable levels of tolerance have been observed across species (Pinkard et al. 2017). As the genus contains more than 700 species there is still much uncertainty regarding the ability of most species, including those being developed for durable timber in New Zealand, to recover from different levels of insect damage. With regard to the newly developing durable eucalypt industry in New Zealand, although some areas in New Zealand have similar climate conditions to Australia, stress by drought and other local conditions, may further alter the tolerance of some *Eucalyptus* species to herbivory in the dryland areas.

## 1.4. Objectives and thesis outline

Understanding the risks and impacts of insect defoliators for *Eucalyptus* is necessary for the development of the emerging durable eucalypt forestry industry in New Zealand and any genetic improvement programmes in eucalypt plantations due to the high biosecurity risk associated with established pests and on-going insect incursions from Australia. Effort put into effective IPM in the initial stage of forestry breeding programmes will provide a strong basis for the future success of the programmes. This thesis seeks to address four primary objectives:

The first objective is to understand the population dynamics of the four major insect defoliators currently present in the dryland eucalypt plantations in the South Island, and develop a degree-day model to predict the phenology of *P. charybdis* in one of these sites. Understanding of pest phenology is essential to determine when pests needs to be controlled to prevent production loss before any outbreaks occur. Knowing the phenology of defoliators and using modelling techniques to predict when a pest population will exceed the tolerable level can provide vital information for the timing of control, increasing pesticide-use efficiency and reducing negative impacts on the environment.

The second objective is to assess the impact of insect defoliation on the growth of young *E. bosistoana* growing in dryland area in New Zealand. This involves investigating the impact of different defoliation severity, timing and repeated defoliation on tree growth, which can provide defoliation threshold information to help forest managers to decide if or when they should apply pesticide.

The third objective is to identify families of *E. bosistoana* that have higher/lower resistance or tolerance to insect defoliation. *Eucalyptus bosistoana*, like other *Eucalyptus* species, is likely to exhibit a large variation in growth properties and susceptibility to insects between individual trees. In a breeding programme, it is important to maintain those individual trees with elite growth and wood properties that also exhibit the greatest pest tolerance/resistance to a range of pests (because tolerance/resistance to just one pest cannot proof against unknown incursions)

while eliminating those that are most susceptible to pest damage. This will improve our understanding in insect-plant interactions in forest plantations and help in selecting suitable breeding stock to reduce current and future biological risk to *E. bosistoana* and the wider eucalypt plantation industry.

As variations in growth, wood properties and insect susceptibility are expected between eucalypt species and within *E. bosistoana*, variation in susceptibility to the newly introduced paropsine beetle, *Paropsisterna variicollis*, may be detected between durable eucalypt species grown in dryland areas. Therefore, the fourth objective of the thesis is to determine if between-species variation in pest susceptibility can be detected to *Pst. variicollis*. This will reveal the variation of insect susceptibility of different *Eucalyptus* species in insect susceptibility, providing information for future breeding selection.

This introduction has presented the values of the emergent dryland eucalypt industry to the future of forestry industry and the environment of New Zealand, and shown the pest situation in *Eucalyptus* forests in New Zealand and the biosecurity risks faced by the industry, as well as indicated how integrated pest management can benefit the development of the eucalypt industry. The following part of the thesis is structured based on the order of the above objectives, and concludes with a comprehensive discussion integrating the findings from each chapter to provide a thorough demonstration on how an integrated pest management programme for dryland eucalypts in New Zealand might work, and offer suggestions on forest plantation pest management in general.

# CHAPTER 2 PHENOLOGY OF KEY INSECT DEFOLIATORS ON *EUCALYPTUS BOSISTOANA* IN A DRYLAND SOUTH ISLAND SITE

## 2.1. Introduction

### 2.1.1. Background

Understanding the population dynamics of insect pests, including lifecycle parameters, voltinism and population size, throughout the year is key to evaluating pest potential and developing monitoring methods for integrated pest management (IPM) programmes. Similarly, prediction of seasonal variability in pest phenology is vital to the application of control practises. A well designed monitoring programme provides valuable information allowing forest managers to implement control measures to avoid outbreaks of insect pests, and to avoid unnecessary pesticide use when pest population size is under economic thresholds. Ideally, devastating production loss can be prevented and impact on the environment can be minimised. Defoliators are the main pest insects found in eucalypt plantations in New Zealand. As some of these are insignificant or even unknown in their native ranges in Australia, information on the population dynamics of these insect defoliators is scarce. The population dynamics of some of the insect defoliators that can cause severe damage to eucalypts in New Zealand have been assessed in the North Island. However, as temperature and rainfall differ substantially on a regional level in New Zealand, it is necessary to study their population dynamics in the local context.

Four of the most common eucalypt insect defoliators in the South Island are *Paropsis charybdis* (the *Eucalyptus* Tortoise Beetle), *Opodiphthera eucalypti* (the gum emperor moth), *Strepsicrates macropetana* (the *Eucalyptus* Leafroller) and *Phylacteophaga froggatti* (the *Eucalyptus* leaf-blister sawfly) (Chapter 1, section 1.1.2). These species are all considered pests to some degree in New Zealand and have been observed damaging *Eucalyptus* in NZDFI sites.

Life cycle and population dynamics of *O. eucalypti* have been reported in White (1972), Alma (1977) and Phillips (1993), but all lack details of how this information was determined. This is likely because it was not considered a significant pest. Two generations were observed in Melbourne and northeast Victoria annually (White 1972). Life history and phenology of *S. macropetana* has been studied in the laboratory in New Zealand ((Mauchline et al. 1999), but field phenology has only been assessed in the North Island. The insect was predicted to have multiple generations annually based on its development time (Mauchline et al. 1999). Studies of *Ph. froggatti* in Australia has shown the insect had overlapping generations with most damage to host plants occurring between autumn and spring (Loch et al. 2004). No studies have been conducted on its population dynamics in New Zealand.

Among these four defoliators, *P. charybdis* is the most important in New Zealand. Historically it has not been a significant pest in Australia, however, it has been identified as a potential future pest in south-eastern Queensland (Nahrung 20016), where plantation eucalypt species are grown outside their natural range. It has been recorded to have one or two generations in Queensland (Nahrung 2006), and overwintering adults emerged from October. The first and

second adult abundance peaks appeared in November and January respectively, while the population with one generation had the adult peak in December. In the North Island of New Zealand, the adult *P. charybdis* populations peaked in January and late March (McGregor 1989). Two generations of *P. charybids* were observed in the central North Island and Nelson in the South Island (Clark 1930, Styles 1970, McGregor 1989).

Insect population dynamics are affected by biotic and abiotic factors. While the influence of biotic factors is significant, including mortality due to predators and parasitism, it is not the main focus in this chapter. In fact, as *Eucalyptus* insect defoliators in New Zealand have arrived from Australia, they have few effective native natural enemies in New Zealand. Phenology is the study of periodic phenomena of the biology of plants and animals, and the impact of climate and season on these phenomena (Stedinger et al. 1985). Phenology modelling has been used to understand the impacts of abiotic factors on insect population dynamics, and to improve pesticide application efficiency by predicting the best time to target specific life stages of insects in the field. One of the most common phenology models is the degree-day model based on thermal accumulation. Instead of using normal time, development time of cold-blooded animals can be measured by degree-days (expressed by the measurement unit °d or DD), which is a phenological time, because temperature plays a major role in their development (Worner and Penman 1983). The degree-day model is based on the assumption that, within certain limits, development rate is a linear function of temperature, which basically contains two parameters: base temperature and the degree-day (DD) requirements for specific life stage(s). The base temperature ($T_0$/°C) is the temperature below which development cannot proceed (Logan 1988). Development rate increases linearly with temperature above $T_0$/°C until an optimum temperature is reached and declines when the temperature exceeds the optimum. One degree-day presents when the daily temperature is one degree over $T_0$/°C. Thus, by knowing base temperature and the total degree-day requirements of an insect pest, combined with local temperature data, we can estimate seasonal volitinism and the approximate timing at which the target life stage appears in the field.

### 2.1.2. Objectives

The key questions to be addressed in this chapter are: 1) What are the population dynamics of the four most common insect pests in a South Island dryland *E. bosistoana* plantation, and how can this information support future monitoring programmes and pest management strategies? 2) For the most important pest, *P. charybdis*, can a simple degree-day model predict its generations and appearance of life stages? To address objective 1, a survey was conducted in one dryland *E. bosistoana* plantation to monitor the field population dynamics of the four common insect defoliators over two growing seasons. The four defoliators were chosen to represent insect groups with different feeding guilds: chewers, leaf rollers and leaf miners, which results in different damage patterns and positions (new/old leaves and upper/lower crown), seasonal occurrence and levels of defoliation during the year or the rotation cycle (Chapter 1, Table 1.1). To address Question 2, a laboratory experiment was conducted to study development rates of the immature life stages of *P. charybdis* under different constant temperatures to find out their base temperature and degree-day requirements to construct a degree-day development model.

This chapter comprises two parts. Part 1 will address the first objective which is to investigate the population dynamics of the four major insect defoliators in a dryland *E. bosistoana* plantation, and part 2 will address objective 2, which is modelling the phenology of *P. charybdis* using a degree-day model.

## 2.2. Objective 1: Population dynamics of key defoliators

### 2.2.1. Methodology

#### *2.2.1.1. Study site*

This study was conducted at an *E. bositoana* plantation site (41°46'28.80"S, 174°7'55.62"E, 64m altitude) located near Lake Grassmere in Marlborough, in the South Island of New Zealand (Figure 2.1). The study trial was in a dryland farm site with annual rainfall of less than 600 mm, coupled with high sunshine hours. The site was previously used for grazing. *Eucalpytus bosistoana* were planted in October, 2010, on a north-facing slope around 64 m a.s.l. At the start of the study, the trees were 5 years old. There were 1750 trees in the trial from 40 *E. bosistoana* families from provenances from Victorian, Australia. The trial was laid out in an incomplete block design with 50 plots on the site, each made up of 35 trees covering most of the 40 families (Chapter 1, section 1.1.2; Appendix 2).

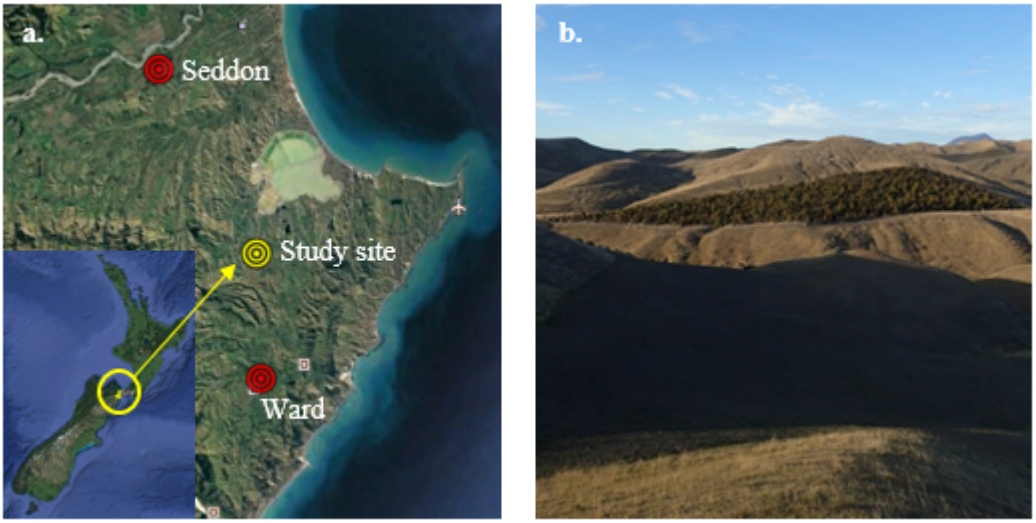



**Figure 2.1** a) Location (yellow circle) of the *E. bosistoana* study site between Seddon and Ward in Marlborough. b) View of stand on the north-facing slope (photo taken in March 2016, when the trees were about 5.5 years old).

#### *2.2.1.2. Climate data*

One EasyLog® (Lascar EL-USB-2) and two Hobo Pendant® (UA-001-08) temperature data loggers were installed at the study site (1 m above the ground in the centre and at the east and west ends of the site, Appendix 2) to record hourly air temperature and humidity. Temperature and humidity were also obtained from a nearby met-station installed in an *E. globoidea* trial

about 3.3km to the NW, along with soil moisture (top 10 cm soil), rainfall, and solar radiation. The met-station measurements were recorded at 2.2 m height. Correlations between temperature data from different loggers and met-station were compared using linear regression analysis.

#### *2.2.1.3. Leaf age composition*

Food quality is a key factor influencing eucalypt insect development and oviposition. The chemical and physical properties of eucalypt leaves, that determine their nutritive quality, change with age (Steinbauer 2001, Hanley et al. 2007). To determine if the presence and/or abundance of the insect defoliators could be related to food quality, the proportions of flush, expanding leaves, and mature leaves (as a proxy for food quality), were visually estimated and recorded for each shoot assessed. Flush included leaf buds and clusters of small expanding current-season leaves at the tips of each shoot; expanding leaves were defined as medium to large current-season leaves still expanding in size; mature leaves were fully expanded and sclerotized leaves either from the current or previous season.

#### *2.2.1.4. Insect survey design*

Pest abundance was monitored for two growing seasons with a sampling interval of 3-4 weeks from November 2015 to March 2016 and from October 2016 to April 2017. One sampling session was missed in November 2016 due to an earthquake preventing access to the site. Up to 233 trees without severe foliage discoloration (221 in November 2015, 229 from December 2015 to early January 2016, 233 in February and March 2016, and 232 in late October 2016 to April 2017) were selected. The same trees and shoots were assessed on each monitoring occasion, with the exception that 14 more trees were added early in the trial (due to the experimental design consideration of the family assessment trial described in Chapter 4 (see section 4.3.1) and 3 trees were eliminated as they became unhealthy (discolouration which may probably due to drought). Trees for assessment were selected across 48 of the 50 plots at the site, avoiding any trees adjacent to those used for the experiment described in chapter 5 (section 2.2) as these were treated with an insecticide soil drench. Three to five shoots (depending on tree size) from each tree from different aspects and positions within the crown (lower, middle and upper) were selected for inspection. As the inspection process was time consuming, each sampling event occurred over 3-5 days. Number of egg batches and larvae of each stage (early, mid, late instar and pupae) were recorded for *O. eucalypti* (adults do not feed and are usually active at night, so they were not recorded). For *S. macropetana*, the number of leaf rolls per shoot was counted and larvae were classified as early, mid and late instar based on head capsule width (early instar <0.5 mm; mid instar 0.5-1 mm; late instar >1 mm), head colour (early instars have black head) and body size according to Mauchline et al. (1999). For *Ph. froggatti,* the number of mines per shoot was counted and the larval stages (early, mid or later instar based on head capsule width) or presence of a pupa/young adult (an egg-shape pupa forms in the mine indicating the individual becoming a pupa or young adult that have not emerged from the mine (Appendix 5-c)) within each mine was determined by shining a light through the leaf. All these life stages of *Ph. froggatti* develop inside a single leaf mine. Only the total number of leaf rolls for *S. macropetana* was recorded in November and December 2015, and only the total number of mines for *Ph. froggatti* was recorded in November 2015. For *P. charybdis* the

number of egg batches and different stages of larval instars were recorded. The number of adults was also recorded as they feed actively on *Eucalyptus* foliage, while the adults of the other three pest species do not. Pictures of these insects are shown in Appendix 3-5.

Five soil emergence traps (Australian Entomological Supplies Pty. Ltd.) were set up on 16 September 2016 to capture *P. charybdis* adults emerging from the soil. Traps were put under five severely defoliated trees as close as possible to the stem. Late instar larvae of *P. charybdis* crawl down to the soil when they are ready to pupate. As such, trees with more defoliation damage were selected for placing emergence traps because they were expected to have a higher probability of having *P. charybdis* adults emerging from the underneath soil. Traps were checked daily on survey dates and when adults were observed in the trap, the date and the number of adults were recorded, and adults were released in the field.

General linear model (GLM) with Quassi Poisson distribution was performed using R for multivariate analyses on the relationships between insect abundance and climate conditions, and between insect abundance and the relative proportion of flush foliage, expanding leaves and mature leaves. R-squared values for GLMs were calculated using function rsq (adjusted R-squared) in rsq R package (Zhang 2018). Climate data used to conduct this analysis were the average values over the 20 days leading up to the sampling event.

### 2.2.2. Results

#### *2.2.2.1. Climate and leaf growth*

Climate data for April 2017 was incomplete and as such excluded from analysis. Correlation between temperature data collected from different loggers was strong ($r^2 = 0.9$, 0.92 and 0.97). On-site average daily temperature was 16 ± 0.3ºC and 16 ± 0.2ºC respectively for season 1 and 2 (one growing season was from September to March). Average daily maximum temperature of season 1 was 26 ± 0.3ºC, 1°C higher than season 2 (Figure 2.2a). Average minimum temperatures for both growing seasons were 9.1 ± 0.3ºC and 9.5 ± 0.2ºC for season 1 and 2 respectively. [1]Average daily relative humidity (RH) was similar for both season: 70 ± 0.3% for season 1 and 69 ± 0.3% for season 2 (Figure 2.2b).

Average daily maximum temperature was higher and average daily minimum temperature was lower at the met-station site 3.3km NW of the study site, indicating a slightly different microclimate, but RH was similar. Rainfall and soil moisture were only available from the met-station site. Total rainfall was higher in season 2 (236 mm) than season 1 (218.6 mm), and total rainfall during winter 2016 was 181.6 mm (Figure 2.2b). Soil moisture was lowest in December in both years, despite greater rainfall in November and December 2016 compared to 2015.

New *E. bosisitoana* flush sprouted from early spring and as a relative proportion it peaked in December in both years (Figure 2.3). The proportion of soft leaves (flush and expanding leaves), which are suitable to stimulate oviposition by *P. charybids*, declined thereafter. Mature leaves made up a greater proportion of foliage between February and April in 2017 compared to 2016, which may have resulted from faster growth stimulated by increased rainfall.

[1] RH was calculated using data from September to February only, because logger failed to record data in March 2016

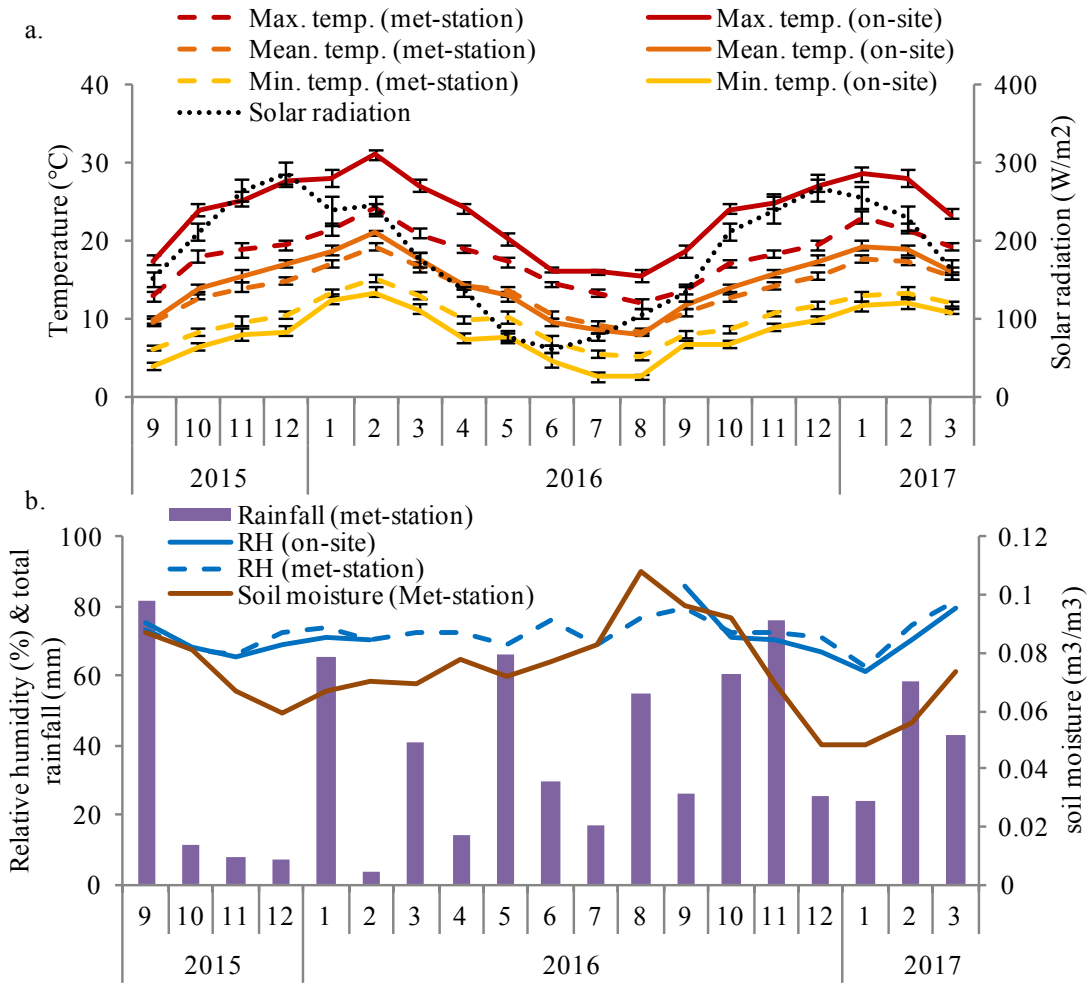



**Figure 2.2** Climate data recorded over the experimental period at the study site and at a met-station 3.3 km to the NW: a) Average daily minimum, mean and maximum temperatures and solar radiation per month; b) Mean daily relative humidity and total rainfall per month, and mean monthly soil moisture measured in the top 10 cm of the soil profile.

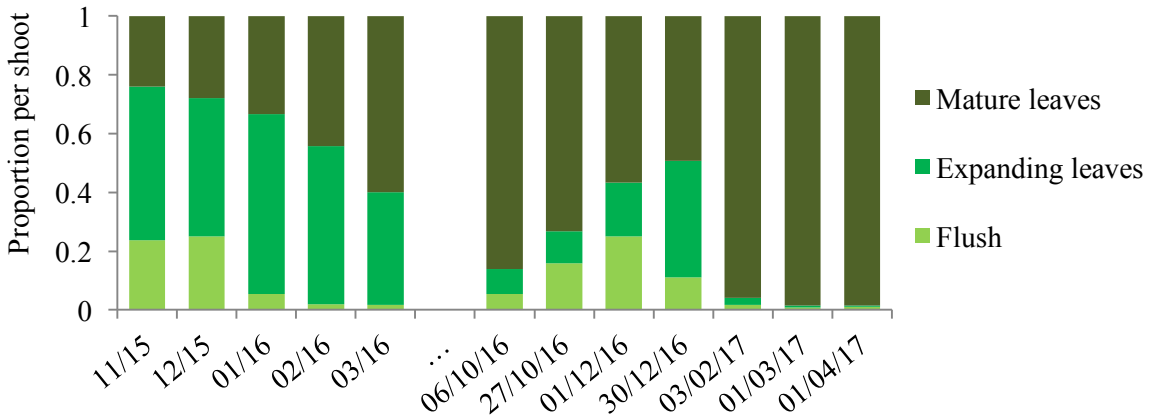



**Figure 2.3** Average proportion of flush, expanding leaves and mature leaves per shoot on each sampling occasion over the two year experimental period. n = 3-5 shoots x 221-233 trees.

### *2.2.2.2. Population dynamics of key insect defoliators*

*Opodiphthera eucalypti*

*Opodiphthera eucalypti* were observed to have overlapping life stages in both seasons (Figure 2.4). Evidence for two loose generations was observed in season one as there were two peaks in egg and late instar larvae abundance. Eggs presented in November, January and February, with peak abundance in November and February. Late instar larvae peaks subsequently appeared in December (first generation larvae) and March (second generation larvae). In January, only small numbers of eggs and late instar larvae were observed, reflecting the end of the first generation and beginning of the second. Two pupae appeared in February, and hatched by the end of the season. In the second season, only one generation was observed. Egg batches presented from early October but some eggs from most of these early egg batches did not hatch. These eggs remained on the leaves and were observed to hatch in late summer. Population peaks (larvae) appeared again in early December but their abundance was about 3 times higher than the first season peak. Pupae were found from early October to April. Most pupae found in October emerged as adults by December, but one pupa found in October hatched just before March.

No significant correlations were found between the abundance of any *O. eucalypti* life stages and recorded climate factors except for the positive correlations between the abundance of pupae and on-site relative humidity ($P<0.05$) and between pupae and soil moisture ($P=0.01$) in the met-station site.

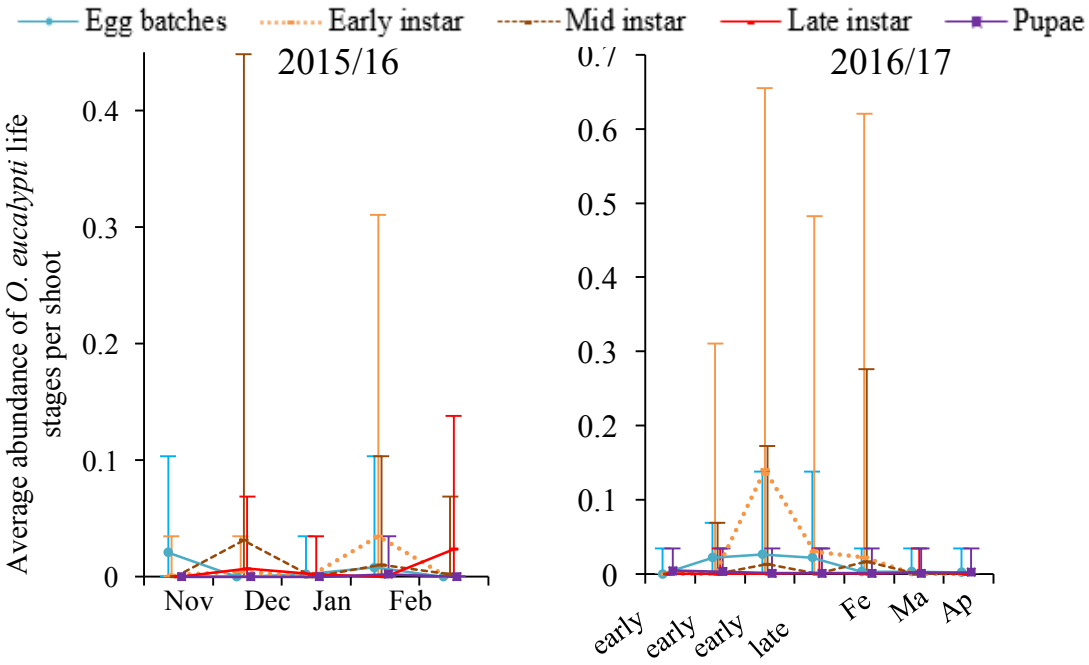



**Figure 2.4** Average abundance per shoot of *O. eucalypti* life stages observed monthly in the field over 2 seasons *n*= 862 (Nov-15), 892 (Dec-15 to Mar-16), 898 (early Oct-16), 909 (late Oct-16 to Apr-17) shoots. Error bars show the 95% confidence intervals.

*Strepsicrates macropetana*

*Strepsicrates macropetana* was observed to have multiple overlapping generations in both seasons, with most immatures stages present throughout the seasons. Eggs of *S. macropetana* cannot be observed easily by eye and adults are not able to feed, so were not recorded. Larval abundance peaks corresponded with the hottest period in both seasons (Figure 2.5 & Figure 2.2a). Early, mid and late instar larvae were all most abundant in February of the first season. In season 2, peak abundance of early instars occurred in late October and late December, while for mid and late instars, peaks were in late December and early February respectively. The peak of late instars abundance in February was bigger than the peak of mid and early instars in December.

There was a significant relationship between the abundance of *S. macropetana* and both maximum on-site ($R^2$=0.56, P=9.40e-05) and met-station ($R^2$=0.80, P=0.002) temperature (Figure 2.6). The relationship between the abundance and mean temperature was marginally significant (P=0.052). Since *S. macropetana* normally live in leaf rolls formed from flush and expanding leaves located in the tops and outer part of the crown, temperatures recorded in the met-station rather than the loggers on site which was only about 1 m from the ground may be better indicators of temperatures experienced by *S. macropetana*. The relationship between the number of leaf rolls and other climate variables recorded on-site and at the met-station were tested, but no significant correlations were found. A correlation between the number of leaf rolls and the proportion of expanding leaves was marginally significant (P=0.053).

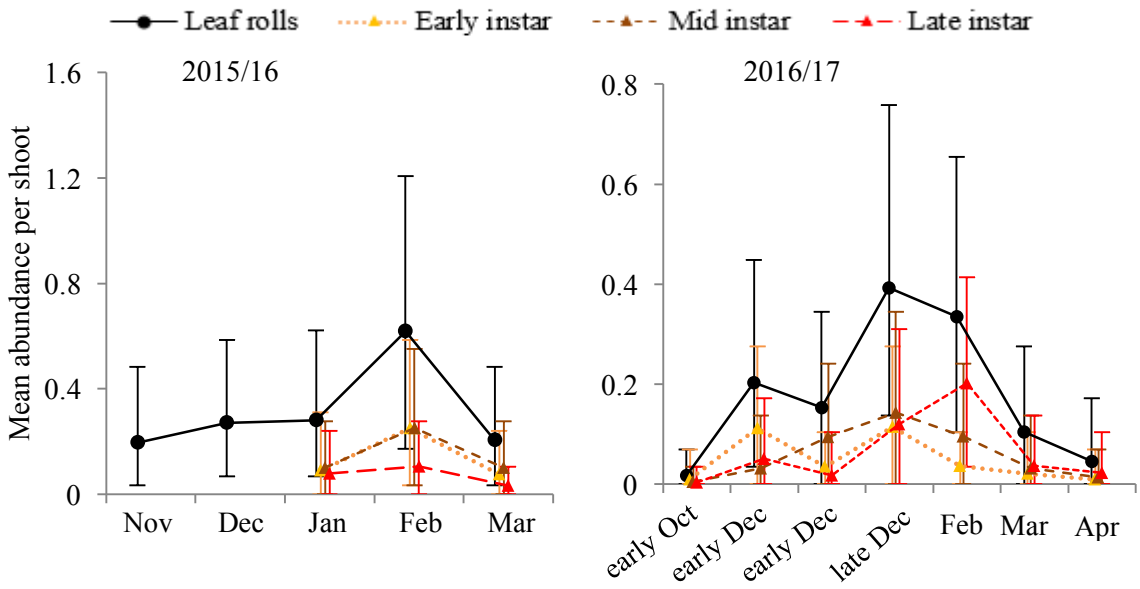



**Figure 2.5** Average abundance per shoot of *S. macropetana* leaf rolls and individual life stages observed monthly in the field over 2 seasons n= 862 (Nov-15), 892 (Dec-15 to Mar-16), 898 (early Oct-16), 909 (late Oct-16 to Apr-17) shoots. Error bars show the 95% confidence intervals.

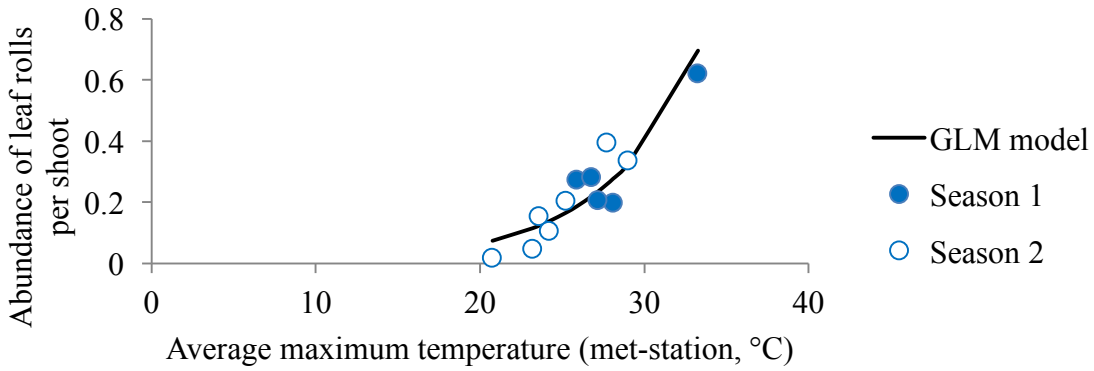



**Figure 2.6** Significantly positive relationship ($R^2$=0.80, P=9.40$^{e-05}$) between maximum temperature recorded in met-station and abundance of leaf rolls containing *S. macropetana* on *E. bosistoana* over 2 growing seasons (Season 1 = October 2015-March 2016, Season 2 = October 2016 – March 2017).

*Phylacteophaga froggatti*

*Phylacteophaga froggatti* was the least abundant insect of the four defoliators studied. Overlapping generations were observed in both seasons. The sawfly appeared to have two population peaks in season 1, but as there was no sampling after March, the size of the second population is unclear (Figure 2.7). The greatest number of mines was observed in December. For season 2, the population of *Ph. froggatti* was somewhat lower. The population increased from early spring and peaked in late December, then dropped in February to a level similar to early spring before increasing again to a peak in early March. The size of the second peak was about half that of the first. Pupae/adults appeared in December and March in season 1 and in late October, December and March in season 2. No significant correlations were found between *Ph. froggatti* abundance and any of the climate factors measured.

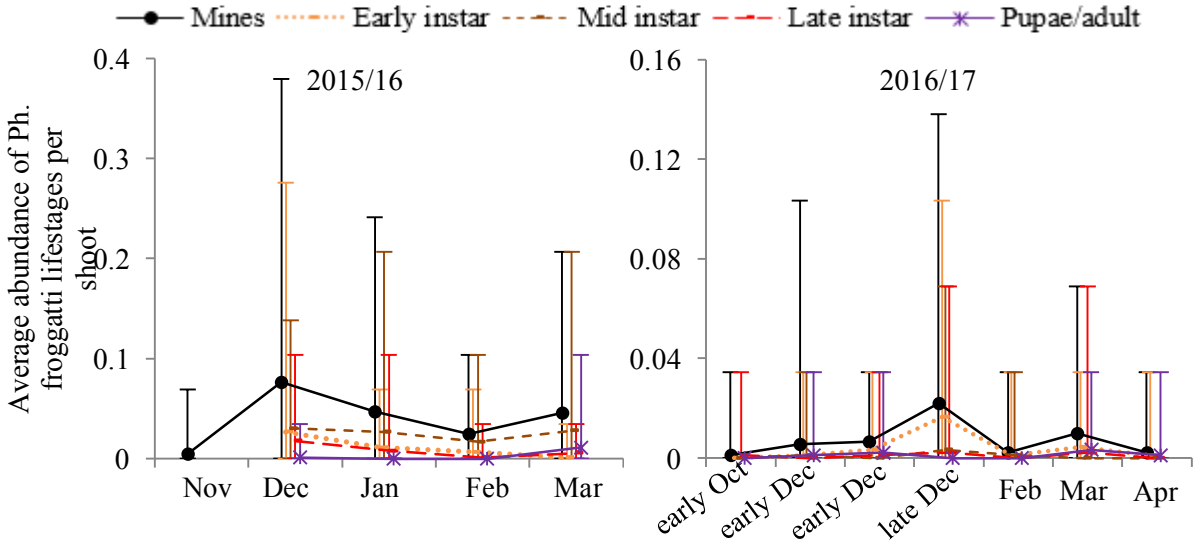



**Figure 2.7** Average monthly abundance per shoot of *Ph. froggatti* life stages observed in the field over 2 seasons n= 862 (Nov-15), 892 (Dec-15 to Mar-16), 898 (early Oct-16), 909 (late Oct-16 to Apr-17) shoots. Error bars show the 95% confidence intervals.

*Paropsis charybdis*

Only one *P. charybdis* generation was observed in each season in the study site (Figure 2.8). Population dynamics in September and October 2015 were not assessed here because of other research activities, and as such the emergence of overwintering adults was missed. In season 1, both eggs and larvae peaked in November. Only 1-2 egg batches were found in December, January and February, and none in March. Early instar larvae (1st and 2nd) dominated in November, after which larval abundance declined. The 4th instars presented at very low abundance throughout the season. Adults were present from November to February, and peaked in January. The first season sampling ceased in March after no *P. charybdis* of any life-stage were found. For season 2, earlier sampling detected eggs, early instars and adults in early October. Fewer egg batches, three in total, were found compared to the first season (13), all in early and late October. Larval abundance (most as early instars) peaked in late October, and 4th instar larvae peaked in early December. No data was collected in November because the site was inaccessible following a major earthquake. No larvae were found from early February. The abundance of adult beetles decreased from late October, but then went up and peaked in late December. One adult was found in an emergence trap in October 2016, and 8 more from 30 December 2016 to 4 January 2017 (Table 2.1), which matched the observed increase in the adult population observed by pest counting on shoots. The adult population went down to a very low level in early February and March and no *P. charybdis* were found by early April.

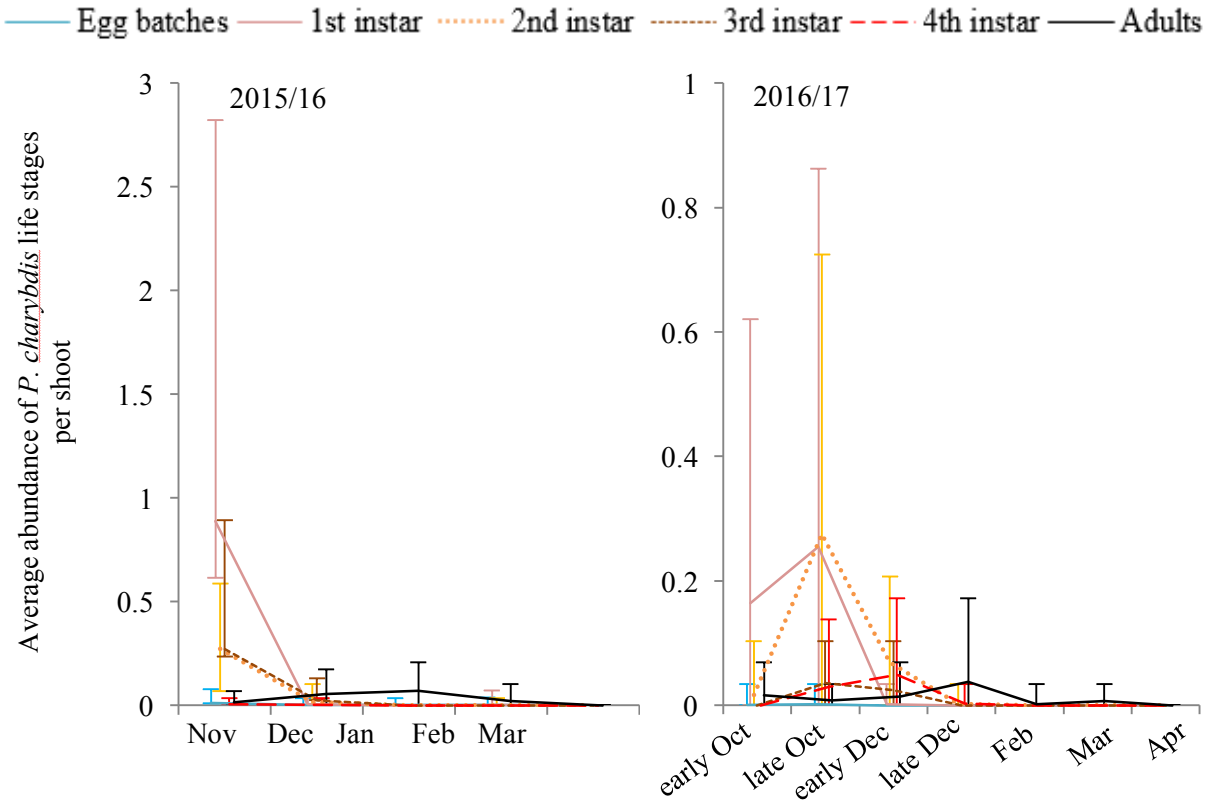



**Figure 2.8** Average abundance per shoot of *P. charybdis* life stages observed monthly in the field over 2 seasons n= 862 (Nov-15), 892 (Dec-15 to Mar-16), 898 (early Oct-16), 909 (late Oct-16 to Apr-17) shoots. Error bars show the 95% confidence intervals.

**Table 2.1** Number of adult *P. charybdis* detected in five emergence traps set from 16 September 2016 to 1 April 2017 under trees heavily defoliated in the previous season.

| Dates checked | Trap | No. adults |
|---|---|---|
| 16-19 Sept. 2016 | - | 0 |
| 6-8 Oct. 2016 | - | 1 |
| 27-30 Oct. 2016 | - | 0 |
| 1-4 Dec. 2016 | - | 0 |
| 30 Dec. 2016 | Trap 1 | 1 |
| | Trap 2 | 2 |
| 1 Jan. 2017 | Trap 1 | 1 |
| | Trap 2 | 1 |
| | Trap 4 | 1 |
| | Trap 5 | 1 |
| 4 Jan. 2017 | Trap 3 | 1 |
| 3-5 Feb. 2017 | - | 0 |
| 28 Feb. - 2 Mar. 2017 | - | 0 |
| 30 Mar. - 1 Apr. 2017 | - | 0 |

For both seasons, peak *P. charybdis* adult abundance correlated with peaks in the proportion of foliage present as expanding leaves (Figure 2.9). This occurred in January for the first season and late December for the second season, and followed the peaks of flush in early December for both seasons. The relationship between adult abundance and the proportion of expanding leaves per shoot was positive and significant ($R^2$=0.47, P<0.01) (Figure 2.10). When this relationship was tested for season 1 and 2 separately, the correlation was non-significant for season 1 (with fewer data points) but significant for season 2 ($R^2$=0.84, P=0.01). The relationships between abundance of different life stages and climate variables (temperature, relative humidity, solar radiation, soil moisture and rainfall) were tested, but no significant correlations were found.

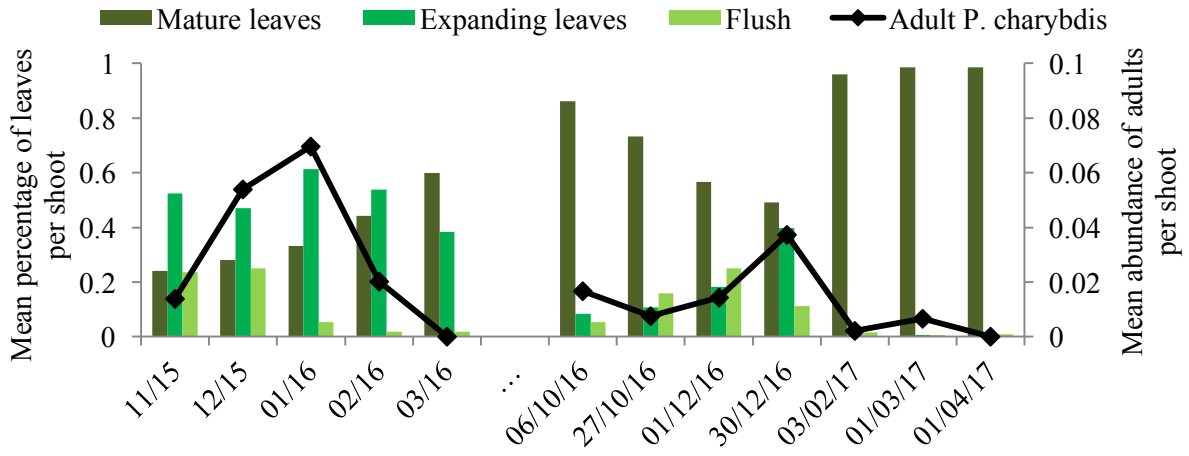



**Figure 2.9** Mean abundance of *P. charybdis* adults relative to the proportion of mature leaves, expanding leaves and flush presented per shoot over two sampling seasons n= 862 (Nov-15), 892 (Dec-15 to Mar-16), 898 (early Oct-16), 909 (late Oct-16 to Apr-17) shoots.

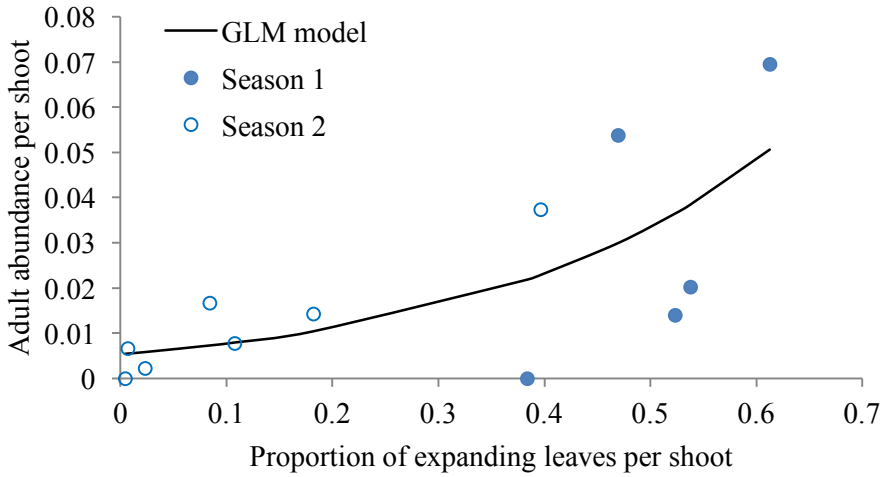



**Figure 2.10** Relationship between adult *P. charybdis* adult abundance and the proportion of expanding leaves per shoot. Values are average values for each sampling occasion. Solid line is the GLM model showing expected values of adult abundance.

### 2.2.3. Discussion

*Population dynamics of* O. macropetana

Among the four common defoliators, *O. eucalypti* and *P. charybdis* share the same external chewing feeding method. Both insect species had 1 or 2 generations in previous studies in Australia or New Zealand (White 1972, Phillips 1993, Nahrung 2006). In season 1, two peaks in egg and larval abundance of *O. eucalypti* were observed in the study site. Although only one pupal abundance peak appeared in season 1, pupae were found in early spring in season 2, indicating that the larvae found in the last survey became pupae by season 2. These results imply that *O. eucalypti* had two generations in season 1. Generations of *O. eucalypti* and *P. charybdis* were not strongly synchronized, but the reasons are different for each species. For *P. charybdis*, it may due to the long life span of egg-laying adults, while for *O. eucalypti*, it is likely due to the development of *O. eucalypti* being highly dependent on local climate (White 1972, Phillips 1993). In particular, the pupal stage of *O. eucalypti* can vary from several months to occasionally 5 years (Phillips 1993). Hatching of eggs within the same egg batches were observed to be poorly synchronized in the study site as well.

The population dynamics of *O. eucalypti* can be affected by a polyhedral virus and larvae may succumb to a parasitic fungus (*Beauveria* sp.) under suitable condition for mycelial growth (Alma 1977). The virus coincident with more rain fall during the second season may have contributed to the higher mortality of *O. eucalpyti*. In season 2, egg hatching was less synchronized than season 1, and late instar larval abundance was much lower compared to the first season, which coincided with more heavy rain events during season 2 (Figure 2.2b). This implies that rainfall may be an important factor in the phenology of *O. eucalypti*.

*Population dynamics of* S. macropetana

Overlapping larval instars throughout the survey period indicates multiple *S. macropetana* generations were present. Mauchline (2000) indicated there were at least 4 generations of *S. macropetana* in their study sites in the Bay of Plenty during a 12-month period. They found winter, spring, summer and autumn generations, and overlapping life stages in the summer suggested a further generation. There was no evidence of a diapause stage for *S. macropetana* (Mauchline 2000) and the full life cycle was relatively short (46.2 ± 11.4 days at 20°C on *E. macarthurii* (Mauchline et al. 1999), with about half of the life cycle duration dedicated to larval stages), so multiple generations can be expected annually in the study site.

Peaks of larval abundance in my study occurred in February in season 1, and in late December and February for early, mid and late instar larvae respectively in season 2. This agrees with findings from Mauchline (2000) which showed the early instars peaked in February and late instars peaked in March in the summer period. Larval abundance peaks also presented in October, December and July in the Bay of Plenty sites, but no such peaks were observed in October and December in my study, and no survey was conducted in July. Differences in the timing of these peaks are likely due to local climate conditions, especially temperature, and also host species, as these factors have previously been found to explain variation in *S. macropetana* abundance (Mauchline 2000). A significant positive correlation between temperature and *S. macropetana* abundance was observed in my study. This strong relationship is likely due to the fact that leaf rolls provide a relatively stable living environment protected from natural enemies and harmful abiotic factors, such as rain and wind, such that the effect of temperature was less influenced by other biotic and abiotic factors. Consequently, temperature can be a good predictor of the abundance of *S. macropetana*.

The higher abundance of late instar larvae in February compared to mid instars in the late December may reflect that larvae of *S. macropetana* are quite mobile. It was frequently observed that leafrollers could easily drop (on a silk thread) to the shoots beneath their original roll to escape danger when the rolls were touched or opened by hand. The short life cycle of *S. macropetana* and strong positive correlation with temperature, may also have allowed new generations to build up quickly between two survey events if occasional high temperature presented during this period. This may imply that more frequent survey interval should be used to catch the highest abundance peak.

The marginally significant correlation between *S. macropetana* abundance and the proportion of expanding leaves may reflect that *S. macropetana* can use both young and mature leaves to build leaf rolls but prefer softer younger leaves. In fact, *S. macropetana* larvae were observed to feed on shoot tips, buds, expanding and soft mature leaves (since large variation of foliage morphology and toughness was observed over different families, and mature leaves of some families were relatively softer) in the study site.

*Population dynamics of* Ph. froggatti

*Ph. froggatti* was the least abundant of the insects assessed. Multiple overlapping generations observed in this study agree with previous studies in Australia and New Zealand (Farrell and New 1980, Faulds 1991, Loch et al. 2004). The only contrast was found in Melbourne, where the blister sawfly has been shown to have well synchronized generations in a 3 years old

*Eucalyptus botryoides* Sm. plantation (Farrell and New 1980). Complete development has been reported to take 6 weeks in the laboratory and 40 days in the field in a *E. botryoides* plantation (Farrell and New 1980). Results from these studies also indicated that most damage from *P. froggatti* in Australia may occur between autumn and spring, similar to the jarrah leafminer *Perthida glyphopa*, (Ohmart 1991). Although no sampling was conducted in autumn and winter in New Zealand, *Ph. froggatti*'s impact is not likely to cause significant damage since leaves are less photosynthetically active during the cold season and sufficient new foliage comes out in the spring to compensate for any losses (Ohmart 1991).

In the second season of this study, the population of *Ph. froggatti* was somewhat lower, which may be due to increased heavy rain events. Rain can be a significant control for *Ph. froggatti*, because water can easily penetrate into the thin covering of the mines (Kay 1986). There are no native natural enemies of *Ph. froggatti* in New Zealand, but the parasitoid wasp, *Bracon phylacteophagus* Austin (Hym.: Braconidae) was introduced from Australia in 1988 (Farrell and New 1980) and has successfully spread to control most populations in New Zealand (Faulds 1990, 1991, Faulds 1993, Withers 2001).

Considering the natural control of *Ph. froggatti*, its damage duration, and its low abundance in this study, it is unlikely that it will become a severe pest in dryland areas. However, when planting in new areas, especially in isolated sites, damage from *Ph. froggatti* could be a problem because the parasitoid population may not catch up with the abundance of *Ph. froggatti* in the first two years of plantation establishment (Faulds 1993).

*Population dynamics of* P. charybdis

According to previous studies from New Zealand and Australia (Clark 1930, Styles 1970, Nahrung 2006), adult *P. charybdis* emerge from early spring, approximately late September. Although only 1 adult was detected in an emergence trap in early October, early instar larvae were abundant at the same time, indicating adults probably did begin to emerge in late September. As peak abundance of eggs was observed in November for the first season but no peaks were observed in November in season 2, the peak of eggs in the second season may have been missed, or the population was too low to notice a peak. Life stages of *P. charybdis* appeared later in this study site than the same life stages appeared in Queensland (Nahrung 2006), but appeared at a similar time as previously observed in the North Island of New Zealand (McGregor 1989, Jones and Withers 2003). The timing at which *P. charybdis* activity ceased in season 2 was consistent with previous studies (April) with the exception that hibernation generally took place from May to June in Nelson (Clark 1930). However, in season 1, no *P. charybdis* were observed in March. The time lag between the observations in the South Island and Queensland, and shorter active period of *P. charybdis* in the South Island indicate, as would be expected, faster insect development in Queensland where temperature is presumably higher. It is also possible that adult *P. charybdis* enter hibernation earlier in New Zealand.

Day length is recognised as a major factor driving insect hibernation (Danilevsky et al. 1970). The hibernation of *P. atomaria* adults (another paropsine beetle) was recognised as photoperiod-induced, with the majority of adults that emerged in late summer entering hibernation after feeding vigorously for several weeks to store energy (Carne 1966). However, because *P. charybdis* activity has been observed occasionally during warm spells in winter

(Dugdale 1965), it is not clear if *P. charybdis* enter a true hibernation or merely quiescence, or if this state is in any way induced by photoperiod. Similar observations have been reported for *P. atomaria*, but they did not feed or oviposit after emerging in the winter. Hibernation of *P. charybdis* has not been observed in the warmest areas of New Zealand (e.g. Port Motueka and parts of North Auckland) (Dugdale 1965), and it is claimed that *P. charybdis* can be reared year-round if fresh foliage is provided (Steven 1973). However, McGregor (1989) also claimed that adult *P. charybdis* collected in the field in summer went into hibernation in the lab even under long day length and warm condition. Thus, it was suggested that the hibernation has been induced in the field, but the trigger for induction and breaking of hibernation is not clear.

Overlapping of population peaks of different immature life stages of *P. charybdis* were observed in the dryland study site, which agrees with historic observations made in two sites in the central North Island and in Nelson (Clark 1930, McGregor 1989). Overlapping life stages may indicate that there are multiple generations per season for the observed insect species, but in this case, it is more likely due to the long life span and extended egg laying period of *P. charybdis* adults. Adults can lay eggs throughout their life (~ three months or more) (Dugdale 1965, Styles 1970), therefore, hatching can occur throughout the season such that different larval instars may be observed at the same point in time. This was observed by Nahrung (2006) who used peaks in the proportion of teneral beetles (pre-ovigenic adults recognised by their softer elytron relative to ovigenic adults) to indicate the appearance of the second generation of adults. The phenomenon of overlapping life stages is also common in other paropsine beetles, such as *P. atomaria* (Carne 1966).

Since different life stages of *P. charybids* occur in the field at the same time, control methods need be effective against multiple stages of *P. charybdis*. Many attempts at introducing stage-specific bio-control agents of *P. charybdis* have been made, but despite two egg parasitoids (*Enoggera nassaui* and *Neopolycystus insectifurax*) becoming established (Murphy 2006, Murray 2010), control is still not sufficient to suppress the population below damaging levels in all regions. Thus, an application has been made to release another parasitoid in 2018 that attacks the larval stages of *P. charybdis* (Withers et al. 2017).

The appearance of only one generation of *P. charybdis* per season in the study site differs from previous observations in the central North Island, Nelson in the South Island and in Queensland, Australia (Clark 1930, Styles 1970, McGregor 1989, Jones and Withers 2003, Nahrung 2006). These regional differences in the population dynamics of *P. charybdis* cannot be explained by any individual factor, and are likely controlled by interactive effects of factors including, temperature, drought, photoperiod and parasitism. Interestingly, in Queensland, both one and two generations were observed in two different study sites (Nahrung 2006), despite the fact both were in locations that are warmer than New Zealand. It is notable that the sampling season in the Queensland study was in an exceptionally dry year, such that lack of new foliar growth may have affected the oviposition and larval development of *P. charybdis*. Drought may have also contributed to the population dynamics observed in the current study. Median annual average temperature and total rainfall at the study site were compared to those of sites used in the earlier North Island and Nelson studies using maps on the National Institute of Water and Atmosphere website (NIWA 2012a, b). It was found all sites have similar median annual average temperatures but the Marlborough site in this study had much lower rainfall (500-700 mm), compared to 1000 mm for Nelson and 1250-2000 mm for the central North Island sites.

*The importance of flush foliage, expanding leaves and drought*

Extremely low rainfall may restrict the abundance of new leaves required to stimulate adult oviposition and larval development of *P. charybdis*. The importance of flush foliage and expanding leaves to paropsines beetles, both for reproduction and development, has been well recognised both from physical and chemical perspectives (Larsson and Ohmart 1988, Steinbauer et al. 1998). This is reflected in the phenological synchrony between the many eucalypt beetles and their host plants, and leaf properties that affect paropsine development, such as leaf toughness and nitrogen concentration. The observed correlation between the proportion of expanding leaves and adult abundance of *P. charybdis* in this study concurs with these earlier findings. Due to selection pressures exerted through pest-host interactions, it is suggested that the pest must evolve and adapt to the phenology of the host to survive. For example Winter Moth eggs hatching to coincide with oak bud-burst is an example of strong selection for phenological synchronization in response to temperature (Visser and Holleman 2001). However, climate change resulting in changes in temperature patterns (warm springs without a decrease in freezing spells) has recently disrupted the synchrony of this particular insect-host interaction.

Such synchrony is not usually necessary in more temperatel climates like Australia and New Zealand, but eucalypts present a possible exception. Numerous eucalypt defences against defoliators include sclerophylly, which some insects have overcome by synchronising egg laying with the appearance of vulnerable flush and expanding leaves (Ohmart 1991). The importance of new leaves in stimulating oviposition and sustaining larval development of paropsine beetles have been stated in several studies (e.g. Ohmart et al. 1985, Steinbauer et al. 1998). There is evidence that leaf chemistry does not inhibit host preference, oviposition, feeding and development of paropsine beetles in the same way it might do for non-eucalypt specialists (Fox and Macauley 1977, Ohmart and Edwards 1991, Ohmart 1991, Cooper 2001). For example, the jarrah leaf miner *Perthida glyphopa* (Lepidoptera: Incurvariidae) readily feeds on *E. marginata* but uses information from chemoreceptors to avoid feeding on oil glands and ovipositing on undesirable leaves (Mazanec 1983, 1985). Other eucalypt feeders have the ability to store, detoxify or excrete harmful plant secondary metabolites after ingestion (Cooper 2001). For paropsine beetles, leaf toughness and nitrogen levels are equally or more important than defensive chemistry (Ohmart et al. 1985, Larsson and Ohmart 1988). For *P. charybdis*, only the late instar larvae can feed on mature leaves because they are physically too tough for younger larvae. Therefore, synchrony between oviposition and the presence of expanding leaves is critical for subsequent larval development. Steinbauer et al. (1998) tested the oviposition preference of the paropsine *Chrysophtharta bimaculata* (Olivier), on *E. regnans* (a preferred host) and *E. nitens* (a less preferred host). They found that when presented with the full range of foliar developmental classes (from immature to expanding to mature leaves), *E. regnans* was preferred, but when only immature and expanding leaves were presented, no difference in host preference for oviposition occurred. Therefore, they argued that leaf toughness is important in female choice of oviposition sites and leaf aging rates are as important as the timing of bud burst and presence of leaf flush to insect phenology. Marsh and Adams (1995) showed a similar trend with *C. bimaculata*. The observation that new leaves aged faster in season 2 than season 1 in this study might have contributed to the smaller *P. charybdis* population in season 2, by limiting the availability of leaves suitable for young larvae.

McGregor (1989) and Ohmart (1991) have also shown that different aged leaves consumed by larvae will later affect adult beetle fecundity, with larvae feeding on a diet of younger leaves having higher fecundity as adults.

The effect of drought on *Eucalyptus* leaf properties leading to a change in paropsine population dynamics has been mentioned in some studies (e.g. Ohmart et al. 1985, Nahrung 2006). *Eucalyptus* growing in the most climatically favourable areas in New Zealand can grow throughout the year (Ohmart and Edwards 1991), but in less favourable environments growth can be restricted to one or two periods a year. Studies on some commercial *Eucalyptus* species found that drought increases leaf nitrogen (Marsh and Adams 1995, Larsson 1998). However, it is argued that paropsines cannot take full advantage of this nitrogen as drought also increases leaf toughness, and tough leaves can be only fed on by late instar larvae and adults. It is also hard to establish a general relationship between insect herbivores and leaf nitrogen because of interactions with other abiotic and biotic factors (Marsh and Adams 1995, Larsson 1998). In dryland areas of New Zealand with long-term drought conditions, *P. charybdis* may perform poorly relative to other areas with higher rainfall. *Eucalyptus* in these regions may therefore suffer lower herbivory as a result, but population monitoring is still necessary due to the uncertainty of the complicated relationship between drought, food quality and other environmental factors.

## 2.3. Objective 2: Thermal requirements and phenology modelling of *P. charybdis*

### 2.3.1. Methodology

#### *2.3.1.2. Adult Beetle Colony*

A colony of adult *P. charybdis* was established and maintained in the laboratory with exposure to natural light and photoperiod throughout the experiment. Beetles were collected from Quarry Park (43°35'56.93"S, 172°34'46.00"E) in Christchurch in late September 2016. The colony was supplemented with additional beetles in October and mid December 2016 from the same source. The colony was initially maintained on a laboratory bench, at ambient temperatures of approximately 18 - 20°C, and later moved to another laboratory with higher temperature of around 22°C in December. Fresh *E. bosistoana* shoots (with both old and new leaves, and stems placed into a bottle with water) were supplied for food and to provide oviposition sites and changed every two to three days.

#### *2.3.1.2. Growth Cabinets*

Developmental assays were conducted in four plant growth cabinets (3 Contherm 620 and 1 Contherm 630 Growth Chambers) at Biology greenhouse area at the University of Canterbury. They were set at 8°C, 15°C, 21°C and 28°C with a photoperiod of 10 light:14 dark. The 8°C, 21°C and 28°C cabinets fluctuated up to ± 1°C while the 15°C cabinet fluctuated up to ± 0.5°C Relative humidity in all cabinets ranged between 50% and 65%. Temperature/humidity loggers (HOBO UX100-003) were placed into each cabinet beside the developing insects.

#### *2.3.1.3. Egg Development*

Fresh egg batches were collected each day from the laboratory colony and inspected under a microscope (Leica, M205C, 10x mag.). Egg batches that were clear and homogenous in colour were used for the egg development assay (number of egg batch replicates: $8^{o}$C $n$=32, $15^{o}$C $n$=33, $21^{o}$C $n$=29, $28^{o}$C $n$=21). Each egg batch was put into a separate Petri dish (Figure 2.12.b), checked daily and the numbers of intact eggs and hatched larvae were recorded. The duration of the egg stage at each temperature was calculated as when 50% of eggs had hatched. Hatched larvae were immediately removed from the petri dishes to ensure they did not cannibalise any remaining eggs.

#### *2.3.1.4. Larval and Pupal Development*

To obtain sufficient replicates, egg batches were collected from the laboratory colony over approximately five days. From the egg development trial, it was found that egg development could be categorized into 5 visible stages (Figure 2.11):

1. Eggs clear and homogeneous in colour;
2. Embryo with two small red dots at one end;
3. Embryo with two small red dots at one end and six black dots (legs) on the other end;
4. Embryo with above features, hairs, and visible body segments;
5. Hatched eggs.

To synchronise egg hatching for the larval development assay, stage 1 and 2 egg batches were put into a growth cabinet (28ºC) or lab (18-20ºC) respectively until reaching stage 3; eggs at stage 3 and 4 were refrigerated at 1- 4°C. When sufficient egg batches reached stage 4, all were moved into a 28°C growth cabinet until hatching. All larvae for the development assay were hatched within 18 hours of each other. Larvae were allowed to feed on their egg shells and darken in colour before being moved onto leaves in plastic cups.

There were 10 replicates for each of four treatments (8°C, 15°C, 21°C and 28°C), and each replicate initially consisted of 20 larvae, placed into one plastic cup. The number of larvae within each replicate decreased over time as not all individuals survived to the next life stage. By the time of pupa stage, number of individuals remained in per treatment were: 1-4 for 8ºC, 12-19 for 15ºC and 15-20 for 21ºC and 28ºC. For the 8ºC treatment, the number of replicates was reduced to eight from the 3$^{rd}$ instar on as all larvae in the other two original replicates died. To avoid maternal effects, each replicate was made up of larvae from multiple egg batches (one larva from each available egg batch). A 15 cm terminal section of an *E. bosistoana* shoot bearing flush foliage was place in each cup. Leaf stems were inserted through a hole in the bottom of the cup into water in a specimen jar underneath. The cup was cover by a piece of net mesh (Figure 2.12.a & b). Fresh leaves of *E. bosistoana* were collected from the Harewood Park nursery (43°28'02.09"S, 172°35'16.40"E) in Christchurch to replenish food every three to five days depending on the leaf quality and amount required.

The larval development assay started from 22 December 2016. Larvae were checked daily and the numbers of individuals that had died or changed from one life stage to the next was recorded. Duration of each life stage was calculated as when 50% of surviving individuals had moulted into the next stage. To prevent over-crowding as larvae got bigger and consumed more leaves at 2$^{nd}$ or 3$^{rd}$ instar, each replicate was separated into multiple cups, so that each contained no more than four to five larvae.

After the 4$^{th}$ instar larvae stopped feeding and became pre-pupae. They were separated from other larvae into cups on a dry wrinkled paper towel. The cups were stacked on top of a specimen jar containing water to maintain sufficient humidity (Figure 2.12.c). Pre-pupae and pupae were maintained on the paper towel until eclosing as adults.

As the 15°C cabinet failed to maintain the set temperature, the treatment was re-started two weeks later than the other three treatments.

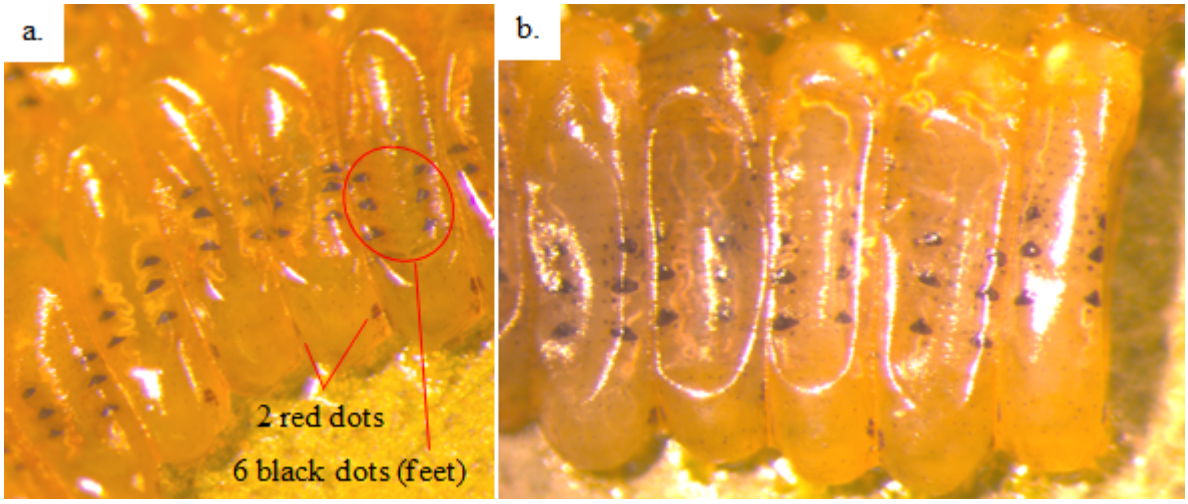



**Figure 2.11** Features of developing *P. charybdis* eggs: a) stage 3 eggs with 2 red dots at one end and 6 black dots (later develop into feet) in the middle; b) eggs in stage 4.

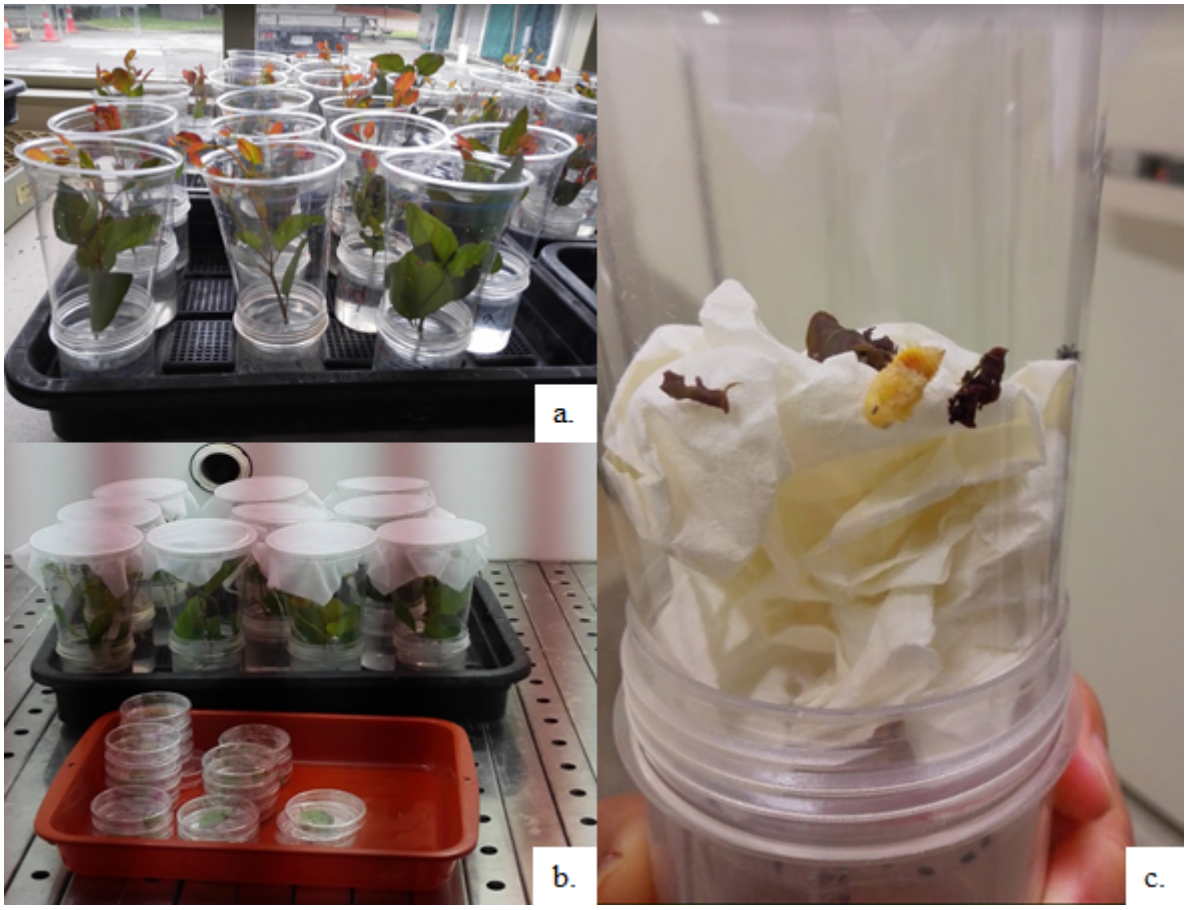



**Figure 2.12** Set-up of *P. charybdis* development study: a) larval rearing cups *containing* fresh *E. bosistoana* leaves with stem penetrating into a specimen jar beneath containing water; b) larval cups (back) and eggs in Petri dishes (front) developing in a growth cabinet; c. pupal development cup.

***2.3.1.5. Estimation of developmental base temperatures and degree-day requirements***

Temperature plays a major role in insect development. Temperature-dependent development of insects can be modelled using either time or rate (Kramer et al. 1991). Degree-day models of insect development are based on the assumption that development rate is a linear function of temperature,

$$y = bx + a \quad (1)$$

Where y is the development rate of the specific life stage of the insect, which is the reciprocal of development time, and x is temperature. Base temperature is the temperature below which the insect would stop growing, and is the x value when y is 0. The degree-day requirement is calculated by 1/b. In many studies on modelling insect phenology using the day-degree model, base temperature and degree-day requirements were estimated using this linear function. However, erroneous predictions may result from using modified rate data instead of observed development time data (Kramer et al. 1991). Consequently, a nonlinear temperature-dependent development model was used here:

$$y = q/(x - T_0) \quad (2)$$

where y is development time of a specific life stage, x is temperature, q is degree-day requirement of this life stage to develop to the next stage and $T_0$ is the base temperature (J.M. Kean, unpublished model, Kean (2015)). Development times of any successive immature life stages can be combined to determine the estimated base temperature and degree-day requirement for the specific life stage duration (for example from 1$^{st}$ instar to pupa).

Degree-day requirements for pre-ovigenic adults (the stage between eclosing as a adults and becoming physiologically capable of laying eggs (see Appendix 6-e) was estimated using information in Edwards and Wightman (1984), where emerged adults did not start laying eggs until the 16th day after emergence at 20 ºC. Thus, the degree-day requirement for pre-ovigenic adult was estimated as:

$$(20ºC - 4.8ºC) \times 15 \text{ days} = 228 ºd$$

The average base temperature of the immature life stages of *P. charybdis* was 4.8°C (see result section). The adult degree-day requirement from emergence to the time 50% of eggs are laid (the median egg laying age) was calculated using information from Styles (1970), where a female *P. charybdis* laid 74 egg batches (1791 eggs) over 123 days, and from older studies (J. S. Dugdale, pers. comm., in Styles 1970) that *P. charybdis* can live for 2-3 months and laid an average of 1783 eggs during this period. Since egg laying is rather uniform throughout the females' life as mentioned in Dugdale (1965), the 46$^{th}$ day was taken as the approximate point that half the egg batches were laid by the female *P. charybdis* in these studies, and it was assumed the experimental temperature, reported only as 'under laboratory conditions', was 20°C. Since the estimation of the median egg laying age was from studies with no detail about exactly how long the lifespan of the adults was, ±5 days was added to the estimation to provide conservative variation around model predictions. Therefore, the degree-day requirement of time to when 50% of eggs were laid by one female *P. charybdis* was:

$$(20ºC - 4.8ºC) \times 41 \text{ days}$$

Or

$$(20^{\circ}C - 4.8^{\circ}C) \times 51 \text{ days}$$

which results in 623.2°d vs. 775.2°d. Both of these degree-day requirement values were examined separately in the modelling process to provide a reasonably conservative estimation.

#### *2.3.1.6. Simulation of* P. charybdis *phenology*

Different model scenarios (combinations of overwintering start dates, hibernation start dates, and estimated degree-day requirements of the median egg laying age) were run to assess the possible number of generations per year and appearances of life stages of an individual of *P. charybdis*. Simulations of *P. charybdis* phenology were conducted in Excel (J.M. Kean, unpublished model, Kean (2015)). A simulation of the phenology of *P. charybdis* was conducted starting from the time the pre-ovigenic adults emergence from the soil. Dugdale (1965) claimed that overwintering adults were rarely fertilised before winter, and generally it is understood that once beetles emerged in spring they fed for several weeks before they are able to lay eggs. Pre-ovigenic adult emergence was set from 1 or 25 September as the start dates of the model for both season 1 and season 2. This corresponded to early spring when adult beetles were first observed at the study site and other sites in the South Island. The on-set of adult hibernation was set to start from 20 March or 5 May respectively based on the observed cessation of *P. charybdis* activity in this (March) and previous studies (April (McGregor 1989 and this study), May (Clark 1930)). On-site temperature data (from 1 September 2015 to 2 April 2017) from a temperature logger installed in a partly shaded spot in the study site was used for model simulation.

Daily thermal accumulation D was estimated by

$$D = [T_{min} - T_0]/4 + [T_{max} - T_0]/4 + [T_{mean} - T_0]/2 \quad (3)$$

Where $T_{min}$, $T_{max}$ and $T_{mean}$ are minimum, maximum and average daily temperature (logging interval = 1 hour). Values in the square brackets were set to 0 if negative (Barlow & Dixon 1980, cited in Kean and Kumarasinghe 2007). This method is more accurate than several other widely-used methods to attain daily thermal accumulation (see Kean (2013) for methods comparison).

Daily thermal accumulation values were added from the start date of each life stage. Once the accumulation value reaches the degree-day requirement (q) of the life stage the insect has accumulated sufficient degree-days to move into the next stage, and daily thermal accumulation would then start again from 0 for the next stage.

### 2.3.2. Results

#### *2.3.2.1. Degree-day Requirements of* P. charybdis

Only 20% of eggs maintained at 8°C hatched successfully (Figure 2.13.a). Egg mortality increased with temperature from 15°C (2.0%) to 28°C (8.9%). Cumulative mortality from 1$^{st}$ instar larvae to pupae was lowest at 21°C (14.5%), while the highest mortality occurred at 8°C (97.5%). Mortality of larval stage at 15°C and 28°C were about 18.5% and 22.5% (Figure 2.13.b). For larval instars, pre-pupa and pupa, mortality was also much higher at 8°C than other temperatures (Figure 2.13.c). Pupae had the highest mortality of all life stages (8°C = 65%,

21°C = 6% and 28°C = 13%). Mortality of 1st instar larvae in 15°C, 21°C and 28°C treatments was the highest and second highest among life stages. In contrast, the 3rd instar had the lowest mortality rates among life stages at 8°C, 15°C and 28°C.

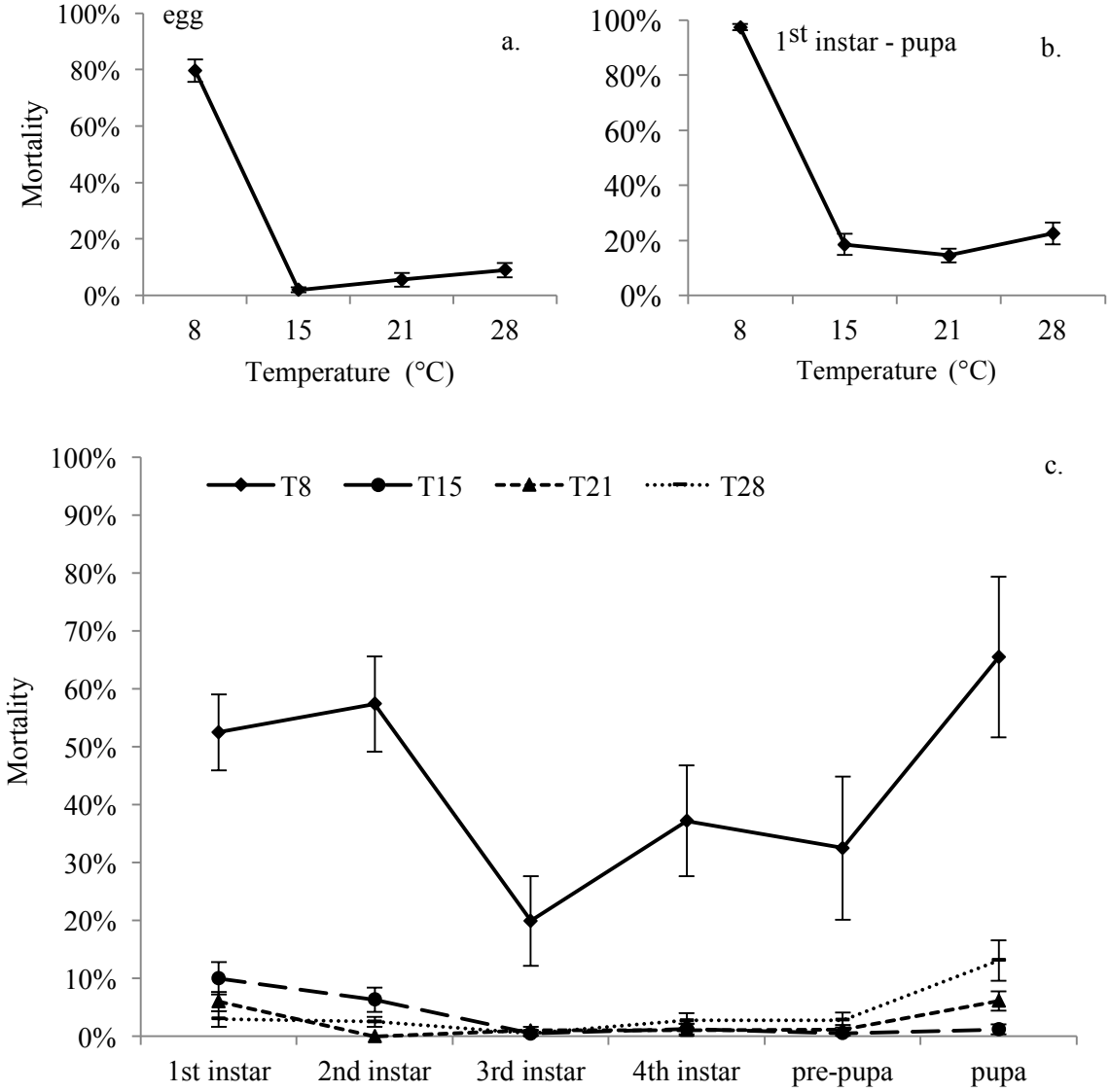



**Figure 2.13** Mean (± SE) % mortality of the different life stages of *P. charybdis* under 4 constant temperatures: a) egg mortality (n=32, 33, 29, 21, for 8, 15, 21 and 28°C growth cabinets respectively); b) Total mortality from 1st instar to pupa (n=10, all temperatures); c) Specific mortality of 1st, 2nd, 3rd, and 4th instar larvae, pre-pupa and pupa (n=10 for all life stages in all temperatures except n=8 for 3rd and 4th instar, and pre-pupa and pupa at 8°C).

As expected, development time was much longer at 8ºC for all life stages relative to 15°C, 21°C and 28°C (Figure 2.14). Non-linear regression used for estimating base temperature ($T_0$) and degree-day requirement (q) for each life stage and the whole juvenile stage (from egg to pupa) is illustrated in Figure 2.15. Development times for 3rd and 4th instar larvae, and for pre-pupa and pupa were combined to reduce error, because one day of data for the 3rd instar and

pre-pupa stages was missing, leading to uncertainty in the 50% transfer date between these two stages. Base temperature was highest for the 2nd instar larvae (5.9ºC) and lowest for the pre-pupal to pupal stage, while 1st instar and late instar stages were similar (5.3 ºC and 5.4ºC respectively) (Table 2.2). Degree-day requirements for eggs and for the combined 1st instar to pupal stage were 84.9ºd and 479.7ºd respectively. Degree-day requirement of pre-ovigenic and ovigenic adults were calculated using information from previous studies (see methodology section), and average base temperature of across all different life stages (egg and 1st instar to pupa) of 4.8 ºC was used for adults. The total degree-day requirement for the development of *P. charybdis* from egg to adult ranged from 1422.7°d to 1574.7°d.

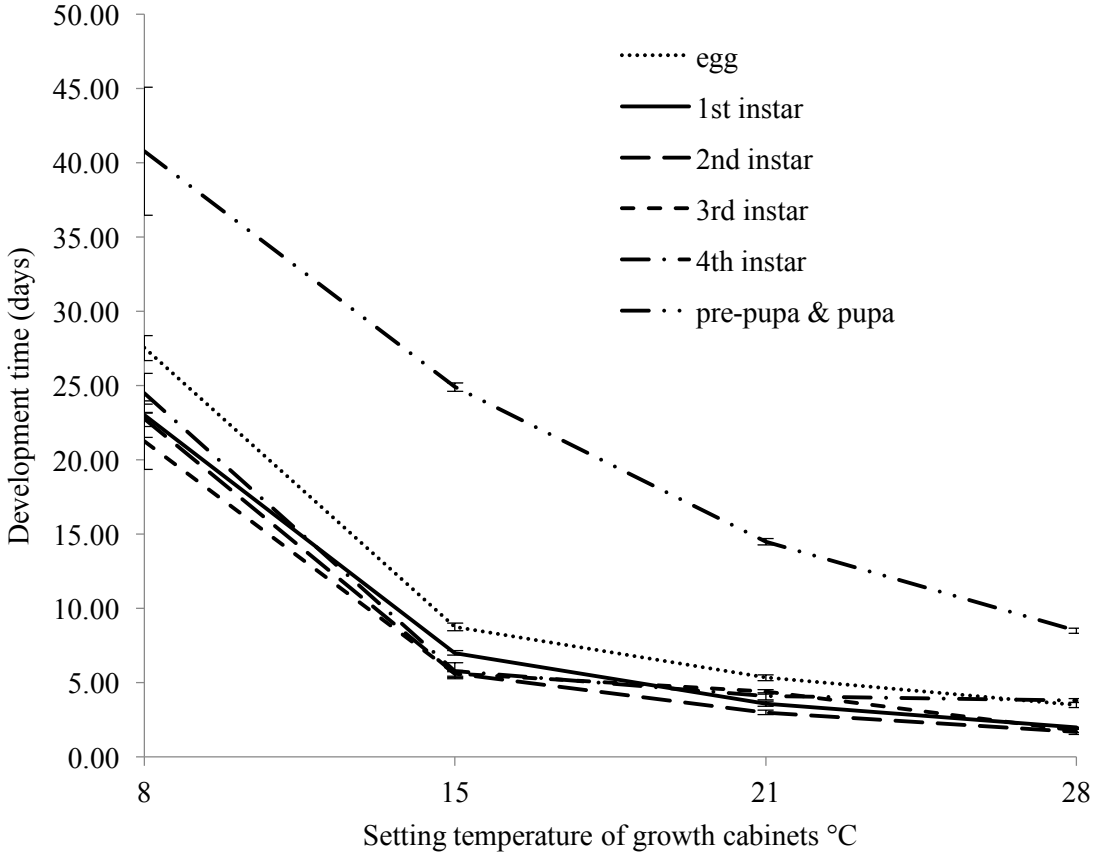



**Figure 2.14** Development duration (mean ± SE) of different *P. charybdis* life stages when reared under constant temperature at 8°C, 15°C, 21°C and 28°C.

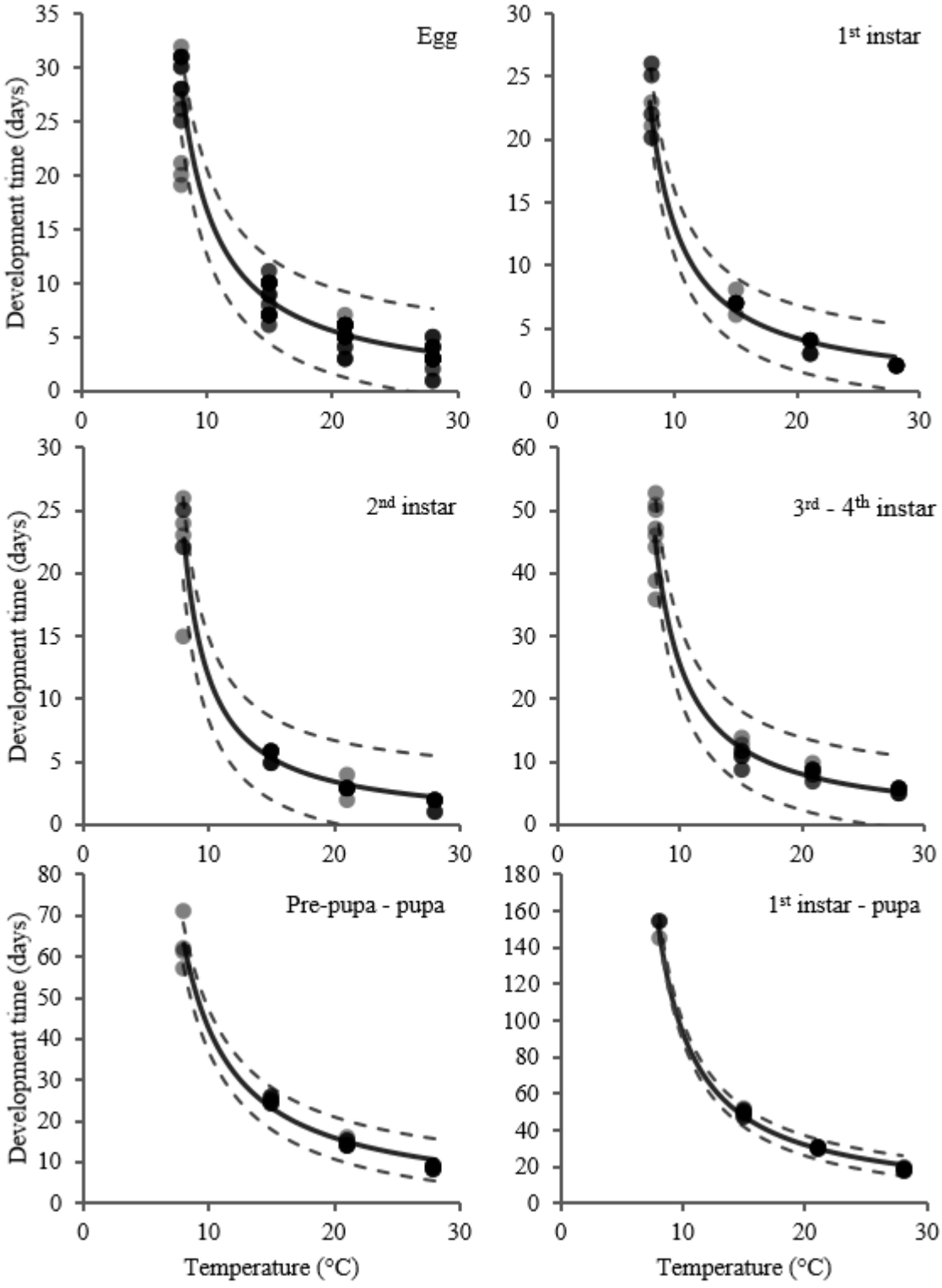



**Figure 2.15** Nonlinear development models for different life stages of *P. charybdis* reared at constant temperatures of 8 °C, 15 °C, 21 °C and 28 °C. Solid lines are the fitted model for the specific life stage, and dotted lines show the 95% confidence intervals. The black dots show the mean development time for each of the 8-10 replicates per temperature.

**Table 2.2** Base temperature ($T_0$) ± SE, degree-day requirement (DD) ± SE, and R-squared and 95% confidence intervals of linear models of different life stages of *P. charybdis*.

| Life stage | $T_0$ ± SE (°C) | DD ± SE (°d) | R-squared | 95% Cl |
|---|---|---|---|---|
| Egg | 4.8 ± 0.2 | 87.8 ± 4.0 | 0.951 | 3.933 |
| 1st instar | 5.3 ± 0.2 | 61.8 ± 4.2 | 0.977 | 2.640 |
| 2nd instar | 5.9 ± 0.2 | 47.9 ± 4.7 | 0.963 | 3.271 |
| Late instar | 5.4 ± 0.2 | 117.5 ± 9.0 | 0.968 | 5.807 |
| Pre-pupa & pupa | 3.9 ± 0.2 | 256.5 ± 10.0 | 0.979 | 5.068 |
| 1st instar - pupa | 4.8 ± 0.07 | 479.7 ± 9.0 | 0.996 | 5.253 |
| Pre-ovigenic adult[1] | 4.8 | 228 | - | - |
| Adult[1,2] | 4.8 | 775.2/ 623.2 | - | - |
| Total[2] | - | 1574.7 / 1422.7 | - | - |

[1] Information estimated from previous studies (see methodology section).

[2] DD for adult stage based on estimated median egg laying age of 41 and 51 days respectively with based temperature 4.8ºC, because ±5 days was added to the estimation to provide variation around model predictions accounting for the uncertainty in the value due to undetailed method description in previous reports on oviposition of *P. charybdis* (see section 2.3.1.5).

#### *2.3.2.2. Modelling the Phenology of* P. charybdis

Using the degree-day requirement and base temperature for each life stage from Table 2.2, the predicted occurrence of each life stage of an individual *P. charybdis* in season 1 and season 2 was simulated. One to two generations per year were predicted by all model variations for season 1 and 2 (Figure 2.16).

Later adult emergence and earlier hibernation dates tended to reduce voltinism to one generation per year. Simulations using the longer degree-day requirement for the median egg laying age (scenarios with the letter L in Figure 2.16), and earlier hibernation start date, predicted one generation (Figure 2.16, scenario c & d), regardless of the date of the overwintering adult emergence. When using the shorter degree-day requirement for the median egg laying age (scenarios with the letter S), only scenario b (25 September as the adult emergence data and 20 March as the hibernation date) predicted one generation of *P. charybdis*. Scenarios assuming the hibernation start date at 5 May, predicted 2 generations of the insect (scenario e, f, g & h). For season 2, scenarios assuming the longer median egg laying age (scenario k, l, o & p) all predicted one generation of the insect. Similarly, for simulations using the shorter degree-day requirement for the median egg age, only the scenario with late adult emergence and early hibernation dates (scenario j), predicted one generation of *P. charybdis*. However, since there was no climate data after 2 April 2017, the predicted durations of the second generation of scenario i, m and n were uncertain.

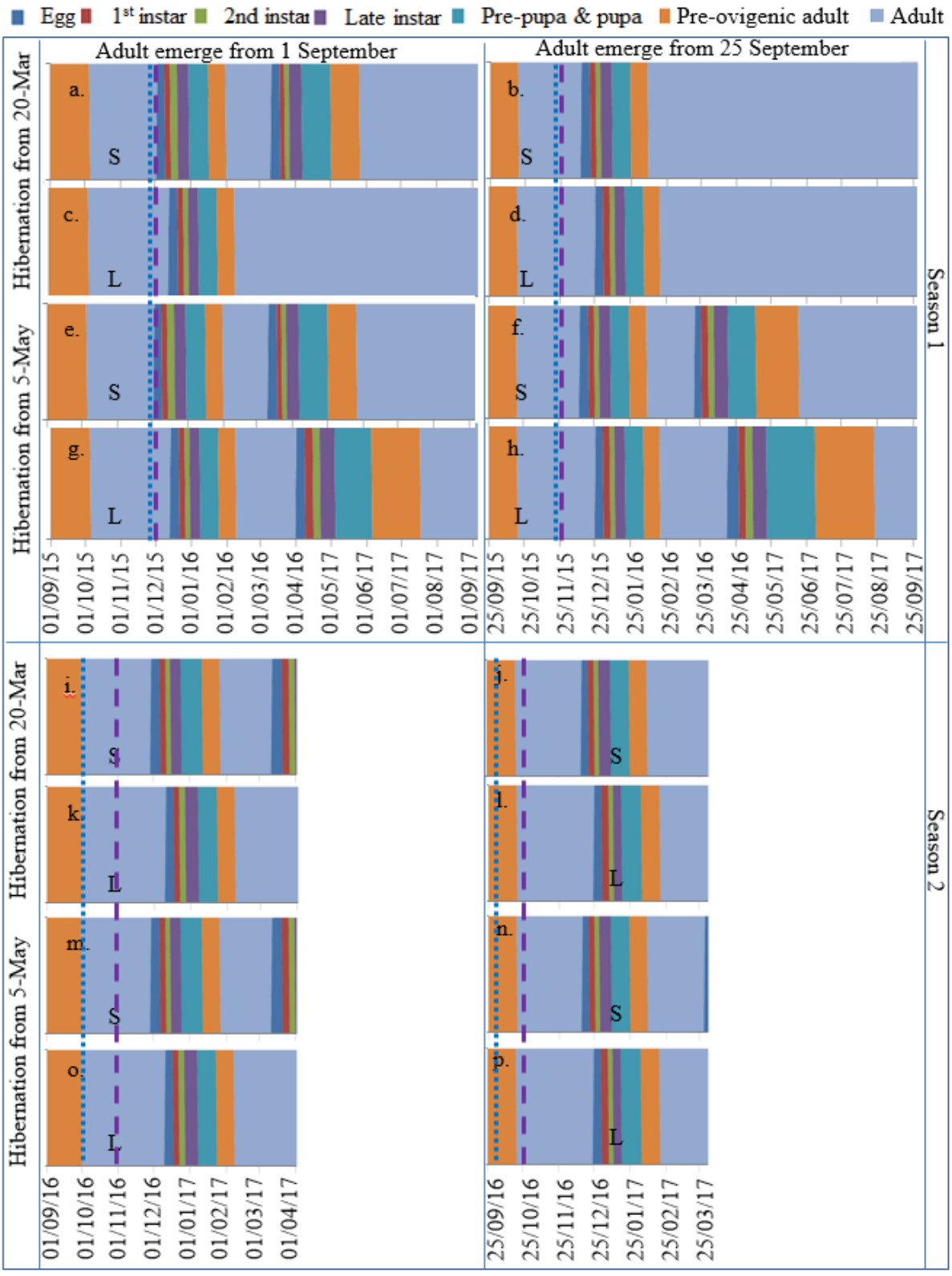



**Figure 2.16** Predicted phenology of *P. charybdis* simulated by the degree-day model using on-site daily maximum, mean and minimum temperature data over two seasons. Scenarios a-p show predicted phenology based on different hibernation start dates (25 March and 5 May), degree-day requirements for the median egg laying age (S= 623.2°d; L= 775.2°d) and overwintering adult emergence dates (1 September and 25 September). Blue dotted lines show the first sampling events in the season that observed egg batches, and purple dashed lines show the first sampling events that observed late instar larvae at the field site (see Figure 2.8).

The model accurately predicted the observed voltinism of *P. charybdis* in 3 of the 8 scenarios in season 1. For season 2, scenario i, m and n predicted 2 generations, but because on-site temperature data was not available after April, it is unknown if other scenarios would predict one or two generations. The scenarios that most accurately predicted the first appearance of eggs (which can be used to predict when they might become damaging larvae) were scenarios a and e (season 1) and these scenarios based on the shorter median egg laying age. However, these scenarios erroneously predicted two generations in both seasons because the assumptions of these models were shorter degree-day requirement for the median egg laying age and an earlier adult emergence date. Scenarios that predicted one generation of *P. charybdis* with the closest predicted first appearance of egg and late instars as seen during field observations were scenario b and c for season 1. The model was a better predictor for season 1 than season 2 respect to appearance of eggs as there was a much greater gap between the predicted and observed egg appearance in season 2. However, the survey started much earlier in season 2 (early October in season 2 but November in season 1), so it was possible to see eggs in October in season 1 if observation started as early as season 2 given the late instar larvae were also present by the time the eggs present.

### 2.3.3. Discussion

To my knowledge, the degree-day approach has not been used to model *P. charybids* phenology as no such models have previously been published. Steven (1973) studied the development duration of the immature life stages of *P. charybdis* but focused on the development duration when fed with different host plant species. McGregor (1989) conducted a similar laboratory experiment to the study here, but base temperatures and degree-day requirements determined for immature life stages were quite different from here. Base temperatures for immature stages ranged from 6°C to 8.1°C, higher than the range 3.9°C to 5.9°C in this study, and degree-day requirements of immature stages were reportedly about half of that observed in this study (303°d in McGregor (1989) vs. 571.5°d in this chapter). These results may be partially explained by the different food sources, regression methods (linear regression using development rate in McGregor's thesis and non-linear regression using development time in my study) and experimental designs. McGregor (1989) used *E. viminalis* (a known preferred and highly suitable host plant (Bain et al. 2009)) but *E. bosistoana* (suitability unknown) was used here. 28°C was included in both studies, but it was eliminated in estimating the development time of 1$^{st}$ and 2$^{nd}$ instars in McGregor's study because the results were lower than expected, and McGregor concluded 28°C was above the optimum temperature for development. There was no evidence for this from my study. As temperatures in the South Island of New Zealand, rarely exceed the optimum temperature of *P. charybdis* predicted in this chapter, optimum temperature was not included in the modelling process. Despite these factors, using the base temperatures and DD requirements from both studies produced similar results. This is because the reductions in base temperature were compensated by corresponding increases in degree-day requirements. Different base temperatures for separate populations of the same insect species have previously been observed in aphids, with Campbell et al. (1974) claiming that the thermal requirement of the insects can adjust to suit the local environment. Compared to *P. atomaria*, which is an important pest in subtropical Queensland where the temperature is warmer than South Island New Zealand, average base temperatures determined for *P. charybdis* were generally lower (*P. atomaria*: 6.4°C (Nahrung

et al. 2008)), and *P charybdis* had fewer generations (*P. atomaria* can have more than two generations per year in some locations in Australia).

Mortality of life stages is an important factor that influences the population dynamic of insects by affecting the population size. Life stages with high mortality imply that these life stages might not require controls since the population will naturally decline. However, control practise is required before *P. chaybdis* reaching to the late instars stage because they produce the most severe damage. Mortality and life span of the resulting adults from larval development essay was not able to be measured in the study, because suitable foliage was not available.

The degree-day model used in this study, predicted voltinism of *P. charybdis* in the field under certain scenarios however further refinement is clearly required. Changing the assumption of the median egg laying age had the greatest effect on predicting voltinism*,* indicating the model is more sensitive to the degree-day requirement of the median egg laying age than to the dates of overwintering adult emergence and hibernation. Although the scenarios that assumed the egg laying age as 775.2°d tended to predict one generation per year, which was consistent with field observations, some results suggested that 623.2°d may be the better estimation. This is because scenarios that most accurately predicted the first appearance of eggs and late instar larvae were those that assumed the median egg laying age as 623.2°d. This estimation of first appearance of life stages is very critical to pest management, because the time when eggs appear in the field determines the start of monitoring. Although scenarios using this assumption tended to predict two generations, the second generation's eggs and larvae may not survive due to increasing frost events from March. However, since no observations were conducted before November in season 1, eggs may have been present earlier than observed and the model predictions for scenario a and e may not be as accurate as they seem.

The model is slightly more sensitive to hibernation start date than overwintering-adult emergence date. As a result, simulations with the assumptions the median egg laying age degree-day requirement as 775.2°d and hibernation starts from late March, were more likely to predict a single generation of *P. charybdis,* as was observed in the field. The model performance is limited by incomplete information of the median egg laying age and start date of hibernation. As the model was most sensitive to the degree-day requirement of the median egg laying age relative to the adult emergence and hibernation start dates, accurate information on the life span of the adult beetles, fertility and fecundity throughout the adult life would markedly improve the models prediction ability. Also, besides pre-ovigenic adults, *P. charybdis* could possibly overwinter as pupae. These stages have been observed to over winter for *P. atomaria* (Carne 1966), though Nahrung et al. (2008) claimed that adults were the only life stage that pass winter. Thus, it would be worth investigating which life stages over-winter and the degree-day requirement from these life stages to the median egg laying age. Similarly, voltinism could be more accurately predicted if hibernation timing could be confirmed, and this information (if there is second generation or not) is critical to control strategies if the first generation has caused above-threshold defoliation (also discussed in section 3.4 in Chapter 3).

Differences between the model predictions and field observation may also have resulted from the influence of factors other than temperature. For example, higher field mortality (especially egg parasitism in late summer), poorer nutrition in the field (due to drought conditions and being limited to a single host species of un-known suitability) and differences between soil and air temperature for pupa development (soil temperature was not measured, so the temperature

experienced by pupae was assumed to be the same as air temperature). Furthermore the model does not have the capacity to account for overlapping life stages. Lifespan and ovigenic period of *P. charybids* adults are very long, so overlapping life stages appeared. Population size and peak life-stage abundance could be more accurately modelled with the above information.

## 2.4. Chapter Discussion and Conclusion

### 2.4.1. Key results from addressing two objectives

1. *Opodiphthera eucalypti* had one to two generations per season. Overlapping of life stages may due to the delay larval development and egg hatch owing to heavy rain events, and the long and variable duration of the pupal stage. Relationships between climate factors (temperature, relative humidity, soil moisture and rainfall) and the abundance of *O. eucalypti* were not significant, with the exception that pupal abundance had a positive significant correlation with humidity and soil moisture.

2. *Strepsicrates macropetana* was the most abundant species of the four common defoliators in the field. Multiple overlapping generations were observed from spring to late summer. Abundance of *S. macropetana* was significantly positively correlated with maximum temperature.

3. *Phylacteophaga froggatti* had multiple overlapping generations from spring to late summer and was the least abundant defoliator among the four species assessed. Populations peaked in December and March in both seasons.

4. *P. charybdis* had one generation per year in the dryland *E. bosistoana* plantation. This differs from previous records in New Zealand and one observation in Queensland that reported two generations per growing season. Climate variables, temperature, relative humidity, soil moisture and rainfall, did not have significant correlations with the abundance of *P. charybdis*. However, peak *P. charybdis* adult abundance over 2 growing seasons was strongly correlated to the proportion of expanding leaves relative to flush and mature leaves.

5. Mortality of *P. charybdis* was highest when reared at 8°C relative to 15°C, 21°C and 28°C. Generally, late instar (3$^{rd}$ and 4$^{th}$ instar) had the lowest mortality among different life stages.

6. Base temperatures of *P. charybids* life stages ranged from 3.9°C to 5.9°C, averaging at 4.8°C. Degree-day requirements of eggs, and larvae to pupal stage were 87.8 ± 4.0°d and 479.7 ± 9.0°d respectively.

7. The degree-day model predicted that *P. charybdis* had one or two generations per year depending on assumptions made regarding overwintering adult emergence dates, hibernation start dates and degree-day requirement of the median egg laying age. The model was most capable of predicting voltinism of *P. charybdis* in the field under the assumptions of longer DD requirement of median egg laying age and hibernation start by 20 March. However assuming shorter DD requirement of median egg laying age, early hibernation start date and later over-winter adult emergent date produced similar results. Predicting the appearance of life stages was not highly accurate, but models that assumed shorter DD requirement for the median egg laying age tended to be the most accurate.

### 2.4.2. Chapter conclusion and discussion

Understanding the phenology and population dynamics of insect pests is vital for their long-term management. The ability to predict seasonal voltinism and the temporal occurrence of different life stages is important for informing effective control measures (Cox 1994). Based on the abundance of *O. eucalypti* and *Ph. froggatti* in the study site, they are not likely to be significant pests in durable eucalypts plantations. Although not usually considered a significant pest, *O. eucalypti* was observed to completely defoliate several smaller *E. bosistoana* trees (around 2 m height) so it may be a severe pest in very young eucalypt plantations, although these trees subsequently recovered their foliage. Generally, *O. eucalypti* has one generation per season (Phillips 1993), but two generations can be found in warmer places (e.g. Melbourne and northeast Victoria (White 1972)). In dryland areas in New Zealand, higher temperatures and dry conditions may favour the development of *O. eucalypti* because they may reduce the virulence of a virus and fungus that naturally control the insect. *Strepsicrates macropetana* was the most abundant pest in the study site and fed primarily on buds and soft leaves, which could reduce the productivity of *E. bosistoana* as more energy is put into replacing foliage. *Strepsicrates macropetana* is not often regarded as a significant pest in New Zealand or Australia (although occasional outbreaks occur). However, as other totricid leafrollers are destructive and economically important, and as *Eucalyptus* are the only food source for *S. macropetana* and are being planted more regularly and widely in New Zealand (Mauchline et al. 2001), the pest status of this insect should be closely monitored within the eucalypt industry. Insecticide may not be a sufficient control if leafroller abundance reaches pest status, because of overlapping generations throughout the year and the protection provided by the leaf rolls. There is no quantitative study that has investigated this insect's impact on *Eucalyptus* tree growth, and although the parasitoid *Trigonospila brevifacies* (Hardy), a leafroller, was introduced to the South Island in 2000, parasitism has not yet been quantified in dryland eucalypts plantations.

Population dynamics of the four insect defoliators assessed in this dryland South Island site were similar to previous observations in Australia and the North Island, except that *P. charybdis* was observed to complete only one, rather than two generations per season. Rainfall may be the vital factor contributing to this difference in voltinism. Lower rainfall and drought may reduce the abundance of expanding leaves and/or change foliar phenology, leading to delayed oviposition and reduced larval performance on less palatable foliage. The important role of expanding leaves was supported by the significantly positive correlation between adult *P. charybids* abundance and the relative proportion of expanding leaves. The degree-day model was capable of predicting voltinism of *P. charybdis*, indicating that with appropriate assumptions and more information on the development rate of pre-ovigenic adults, the model may be useful for assessing the risk of multiple generations of *P. charybdis* occurring in regions where eucalypt plantations have not previously been established. As degree-day models have been used to accurately predict the appearance of life stage of another paropsine beetle, *Paropsisterna agricola* (Nahrung 2004), there is potential to predict the appearance of *P. charybids* life stages using the current model if the DD requirement of pre-ovigenic adults is determined. Predicting egg appearance could be used as an indicator for the start of pest monitoring, to gather information that can then be used to help determine if and when to apply pesticide control.

The fact that results of simulations using the base temperature and degree-day requirements from both this study and McGregor (1989) were similar, may imply that development rates of *P. charybdis* larvae feeding on *E. bosistoana* are similar to those feeding on *E. viminalis,* which

is a known preferred and suitable host plant. However, since foliage of *E. bosistoana* used in the insect development assay was collected from a nursery in Christchurch which had irrigation, and the insects were always fed a combination of expanding and mature leaves, foliar nutrition and the availability of soft *E. bosistoana* leaves in dryland areas are possibly lower than experienced in the lab. As such, *E. bosistoana* may be less suitable for the development of *P. charybdis* in the field.

# CHAPTER 3 GROWTH IMPACTS OF SIMULATED INSECT DEFOLIATION ON *EUCALYPTUS BOSISTOANA*

## 3.1. Introduction

Defoliation by insects and disease is a common cause of reduced tree growth which traditionally may necessitate pesticide application to prevent or stop continuous damage. Understanding the defoliation level that results in damage above an acceptable economic threshold and determining the most appropriate timing for control can increase efficiency and reduce the use of pesticide. Studies have shown that growth rates of several *Eucalyptus* species have the ability to recover from light to moderate levels of defoliation, and for some species growth of defoliated trees can even catch up with that of undefoliated trees. Research on defoliation impacts are necessary to understand the recovery ability of different *Eucalyptus* species and determine damage thresholds below which control is not actually necessary to achieve required growth increases. Forest owners are increasingly encouraged to implement more sustainable pest management techniques, such as improving the efficiency of insecticide use, to obtain sustainable forest certification (e.g. Forest Stewardship Council (FSC) certification (Lemes et al. 2017)) that increases access to certain markets.

### 3.1.1. Measuring the impacts of defoliation

Defoliation studies assessing reductions in growth have generally been limited to a small number of commercial tree species. Kulman (1971) reviewed insect defoliation effects on tree growth but focused mainly on softwoods. Studies specifically on *Eucalyptus* growth and defoliation have been conducted mainly in Australia in the last two decades (see Appendix 1). In these studies, the main defoliation agents include insects (e.g. Pinkard et al. 2006a, Rapley et al. 2009, Loch and Matsuki 2010), *Mycosphaerella* leaf disease (MLD) (Lundquist and Purnell 1987, Carnegie and Ades 2003, Wardlaw 2004), and mammals (Bulinski and McArthur 1999). Artificial defoliation to simulate insect defoliation has also been conducted (e.g. Candy et al. 1992, Elek 1997, Barry and Pinkard 2013). Defoliation studies have focused on a very limited number of widely planted *Eucalyptus* species: *E. nitens, E. regnans* and *E. globulus.* As the genus *Eucalytups* contains more than 700 species, there is much uncertainty around species-specific responses to particular levels of damage. Severity (proportion of leaf area removed), timing (time of year when foliage is lost) and frequency (how many times trees are being defoliated) are the main factors that affect the impact of defoliation on *Eucalytpus* growth. Some studies have also investigated mitigative silvicultural practises (such as fertiliser) that can accerlerate tree growth after a defolaition event (e.g. Pinkard et al. 2006a, Pinkard et al. 2011b, Barry et al. 2012), but this is beyond the scope of this thesis. Although pruning and mammal browsing can also be considered to be defoliation, they may have larger and different effects on trees compared to insect defoliators because they remove whole branches or shoots.

The effects of defoliation severity vary depending on the defoliation agent. Generally, when MLD is the defoliation agent, low levels (10% - 35%) of defoliation have been shown to cause significant reductions in tree height and stem diameter growth. In an extreme example, <10%

of foliage infected by MLD significantly reduced height and diameter growth of 2-year-old *E. globulus* over about two years in Victoria, Australia (Carnegie and Ades 2003). Defoliation by insects has been shown to negatively affect growth of some *Eucalyptus* species, regardless of the duration of attack (acute or chronic) (Matsuki et al. 2007), but the severity that can affect tree growth can differ between species. For example, while as little as 10% insect defoliation significantly reduced DBH and height growth of 3 years old *E. globulus* in the absence of fertiliser (Pinkard et al. 2006a), 60% insect defoliation was sustained before a significant effect was observed on the growth of 2 years old *E. nitens* (Rapley et al. 2009). In a study testing the impact of natural insect damage by defoliators on four *Eucalyptus* species, there was no significant relationship between insect damage severity and seedlot growth, except for *E. dunnii*, although the relationship was not strong, explaining only 40% of the variance (Farrell and Floyd 2007a). There is also evidence that defoliation impacts on trees of different ages can vary (Pinkard et al. 2015). For example, 75% defoliation of the crown length did not significantly affect *E. globulus* seedling growth (Quentin et al. 2012), but 50% defoliation significantly affected the growth of 4 years old trees (Quentin et al. (2011).

In temperate regions, 75% of key plantation pests are defoliators (Eyles et al. 2013b), and most of these are insects. As mentioned above, variable levels of tolerance by *Eucalyptus* species to insect damage have been observed (Pinkard et al. 2017), but this information is rarely used in deciding if chemical control should be applied to control pests. In many cases, the decision to apply chemical controls for insect pests in New Zealand is based on unquantified observations of high pest abundance with little information on how the pest abundance correlates to either subsequent defoliation or impact on growth. Thus, if a threshold level of defoliation below which insect control is not necessary can be determined, insecticide use could be reduced or even avoided. The population dynamics and phenology of specific pest species usually have a pattern on specific host species in specific locations, but severity and frequency of defoliation events caused by them can differ from year to year. A good knowledge of the effects of defoliation severity, timing and repeated defoliation on tree growth is required to determine the control threshold.

Although artificial defoliation (manual removal of leaves using scissors or other cutting tools) cannot completely simulate the impacts of real insect damage, it has been used in the majority of recent defoliation studies because it is technically more feasible and ensures variables, such as defoliation severity, are controlled. Quentin et al. (2010) examined the differences in tree growth and physiologcial responses of potted *E. globulus* seedling to artificial defoliaton vs. real defoliation by *Paropsisterna agricola* (Chapuis) (Chrysomelidae: Chrysomelinae) in a glasshouse setting. Results showed that artificial defoliation could under-estimate the effect of insect defoliation on tree growth, but it did reflect the direction of the responses. However, results from artificial defoliation studies have not been consistent across the few common plantation *Eucalyptus* species assessed. Generally, artificial defoliation only affected tree growth when leaf area loss was above ‘medium’ levels or even at a ‘severe’ level (Candy et al. 1992, Elek 1997). In some exceptional cases artificial defoliation as low as 25% could reduce tree growth (Abbott et al. 1993, Pinkard et al. 2006b). Disagreement also exists on the different impacts of defoliation on tree height growth versus stem diameter growth. Some studies have shown height growth tends to be more affected by defoliation (Candy et al. 1992, Collett and Neumann 2002) but others show stem diameter growth is affected more than height growth (Quentin et al. 2010, Quentin et al. 2011).

Studies agree that disbudding and repeated defoliation (even at low levels) can aggravate the impact of defoliation on tree growth (e.g. Candy et al. 1992, Elek 1997, Candy 2000b).

Removing foliage (excluding apical leaves) from the upper 50% of the crown had a greater impact on tree growth than removing leaves (excluding apical leaves) from the lower 50% of the crown (Collett and Neumann 2002, Pinkard et al. 2007a, Pinkard et al. 2007b). Elek and Baker (2017) argued that timing and frequency were the critical to the impact of defoliation on growth, but few studies have assessed these effects quantitatively. Both Elek and Baker (2017) and Candy (2000b) found that late summer/autumn defoliation had greater impact on tree growth than spring defoliation. There is evidence that repeated outbreak events reduced growth of *Eucalyptus* to a larger extent than a single outbreak event (Collett and Neumann 2002, Elek and Baker 2017).

The underlying physiological responses of *Eucalyptus* following defoliation have also being studied mainly in Australia. The recovery ability of *Eucalyptus* species is attributed to an increase in the photosynthetic rates of the remaining foliage (known as up-regulation), unchanging ecosystem respiration and increased solar radiation transmissivity (Pinkard et al. 2011a, Elek and Wardlaw 2013). While the complexity of biotic and abiotic effects on tree growth responses remains uncertain, modelling is a promising method to predict tree growth, physiological responses and productivity under the combined impact of these factors. Unfortunately, no model can account for the whole range of tree physiological response to insect pest damage (Pinkard et al. 2011a). A comprehensive review on *Eucalyptus* physiological responses to pest attack is in Eyles et al. (2013a).

Due to the complexity of different combinations of defoliation severity, timing and frequency, as well as different tree species and insect pest species, it is difficult to determine the threshold level of damage a tree can sustain before pest control is required to maintain economic productivity. When making genetic selections from breeding stock to improve a new plantation forestry species, it is essential to assess the impact of insect defoliation within the context of the local environment with a suitable defoliation method. Pest management decisions need to be made based on the performance of whatever species/genotype is selected for planting. This is particularly relevant in the selection and improvement of new eucalypt species, such as *E. bosistoana*, for growing in New Zealand's dry regions because interactions between defoliation and drought stress factors can alter tree responses (Coyle et al. 2005). There is evidence that defoliation, although usually not causing mortality, can increase stress caused by drought (McDowell et al. 2013). However, most of this evidence is from studies that examined the effects either not in field trials (usually seedlings in pots) or with only a small number of replicates (Appendix 1). A field trial with high replication is essential to conclusively understand these effects.

### 3.1.2. Integrating defoliation assessment into pest management

To understand the impact of defoliation on tree growth is also important to improve our ability to predict productivity (Coyle et al. 2005). Integrated pest management (IPM) combines a suite of pest management techniques that are effective and environmentally friendly to prevent or reduce pest damage below an economic threshold in the long-term (Kogan 1998). Control and prevention techniques in an IPM programme, such as pest monitoring and assessment, silviculture and control (including biological and chemical methods), are used to regulate damage to a level below this economic threshold. Thus, determining this threshold is crucial for an effective IPM strategy.

Forestry Tasmania has developed an IPM strategy for chrysomelid beetles in *Eucalyptus* plantations (Wardlaw et al. 2010). Part of the strategy is based on Candy (2000a, 2000b, 1992) which integrated insect defoliation impacts into a tree growth model. They used artificial defoliation method to assess the impact of severity, timing and frequency of defoliation on tree growth and to quantify the relationship between defoliation levels and insect population size. The IPM involves monitoring the insect population from November, and if the population is found to be over a specific threshold (eggs/larvae per occupied shoot > 2.4, and ratio eggs/larvae > 1) monitoring will be conducted again 2 weeks later. If the population is not over the threshold, monitoring will be stopped. Control options will be considered based on the second monitoring result. The population thresholds can be changed slightly depending on previous season insect damage and if plantations are suffering from chronic thin crowns. A cost-benefit analysis of the programme found this IPM to deliver a net benefit of $412,492 in 2009-10 in the managed estate (Wardlaw et al. 2010).

### 3.1.3. Objectives

The key questions to be addressed in this chapter are: 1) How do different levels of defoliation affect the growth of *E. bosistoana* in dryland areas? 2) Does defoliation timing affect the impact of defoliation of *E. bosistoana*? 3) Do repeated defoliation events have greater impact on tree growth relative to a single defoliation event? 4) Can defoliated trees recover from defoliation to the point where their growth is equivalent to that of undefoliated trees? To answer these questions, a defoliation trial was established in a 5 years old dryland *E. bosistoana* plantation in the South Island, to investigate the impact of simulated insect defoliation on tree growth. As *E. bosistoana* has not previously been grown on a commercial scale, its ability to recover from insect defoliation has not been studied before. Furthermore, there have been no studies of defoliation impacts on commercial plantation eucalypts in dryland environments. Knowing the recovery ability can help fill the knowledge gap that exists with regard to the complexity of the relationship between *Eucalyptus* species and their insect pests, and the specific performance of *E. bosistoana* under pest stress in dryland conditions. This will help forest managers make decisions on pest management, particularly in deciding the injury threshold to inform the timing and the necessity of applying insecticide.

## 3.2. Methodology

### 3.2.1. Study site

See detail in section 2.2.1.

### 3.2.2. Experimental design

142 *E. bosistoana* trees of similar height, crown shape and competition condition (i.e. surrounded by neighbouring trees on all sides) were selected in September 2015, pruned up to 50 cm above ground level and randomly assigned to one of 7 treatments. These trees comprised 23 *E. bosistoana* families, because there were not enough of any individual family for a fully replicated trial. To control for inherent growth differences between these families, one to two trees from most families were assigned to each different treatment (Table 3.1). Three levels of

defoliation severity were applied: no defoliation, moderate defoliation (approx. 50% of tree crown) and severe defoliation (approx. 90% of tree crown). There were also three defoliation timings: spring defoliation and late summer defoliation, which were single defoliation events, and spring plus late summer defoliation, which were repeated defoliation events. Thus, there were seven treatments in total (Table 3.2): control (C), moderate defoliation in spring (Sp50), severe defoliation in spring (Sp90), moderate defoliation in late summer (Ls50), severe defoliation in late summer (Ls90), moderate defoliation in spring plus late summer (SpLs50) and severe defoliation in spring plus late summer (SpLs90). Defoliation was conducted in October 2015 (spring defoliation) and March 2016 (late summer defoliation), and each defoliation event was completed over a period of 2 weeks.

**Table 3.1** Number of trees per *E. bosistoana* family assigned to each of 7 defoliation treatments.

| Family | C | Sp50 | Sp90 | Ls50 | Ls90 | SpLs50 | SpLs90 | Total |
|---|---|---|---|---|---|---|---|---|
| 101 | 1, | 1, | 1, | | | | | 3 |
| 102 | 1, | 1, | 1, | | | | 1, | 4 |
| 103 | 1, | 1, | 1, | 1, | 1, | 1, | 1, | 7 |
| 104 | 1, | 1, | 1, | 1, | 1, | 1, | 2, | 8 |
| 106 | 1, | | | 1, | 1, | 1, | 1, | 5 |
| 107 | 1, | 1, | 1, | 1, | | 1, | 1, | 6 |
| 108 | 1, | 1, | 1, | 2, | 2, | 1, | 1, | 9 |
| 109 | 1, | 1, | 1, | 1, | 1, | 1, | 1, | 7 |
| 111 | 1, | 1, | 1, | 1, | 1, | 2, | 1, | 8 |
| 113 | 1, | | | 1, | 1, | | | 3 |
| 114 | 2, | 2, | 2, | 2, | 2, | 1, | 1, | 12 |
| 115 | 1, | | | 1, | 1, | 1, | | 4 |
| 116 | 1, | 2, | 2, | 2, | 2, | 1, | 1, | 11 |
| 118 | 1, | | | | 1, | 1, | 1, | 4 |
| 119 | 1, | 1, | 1, | 1, | 1, | | 1, | 6 |
| 120 | 1, | 1, | 1, | 1, | 1, | | | 5 |
| 121 | 2, | 1, | 1, | 1, | 1, | 2, | 2, | 10 |
| 123 | 1, | 1, | 1, | 1, | 1, | | | 5 |
| 124 | 1, | | | | | 1, | 1, | 3 |
| 127 | 1, | 1, | 1, | | | 1, | | 4 |
| 128 | | | | | | 1, | 1, | 2 |
| 129 | 1, | 1, | 1, | 1, | 1, | 1, | 1, | 7 |
| 130 | 1, | 2, | 2, | 1, | 1, | 1, | 1, | 9 |
| Total n | 24 | 20 | 20 | 20 | 20 | 19 | 19 | 142 |

**Table 3.2** Treatments applied in artificial defoliation trial.

| Treatments | Severity | Timing | |
|---|---|---|---|
| Control | no defoliation | | |
| Sp50 | moderate | spring | (single defoliation) |
| Sp90 | severe | spring | (single defoliation) |
| Ls50 | moderate | late summer | (single defoliation) |
| Ls90 | severe | late summer | (single defoliation) |
| SpLs50 | moderate | spring + late summer | (repeated defoliation) |
| SpLs90 | severe | spring + late summer | (repeated defoliation) |

Tree height and stem diameter at 1 m was measured at 4-weekly intervals, using an extendable height pole and digital callipers respectively, from September 2015 to March 2016 and from September 2016 to April 2017 (one measurement was missed in November 2016 due to an earthquake preventing safe access to the site and another was missed in February 2017 due to time restrictions). Percentage increase in stem diameter and height between each consecutive measurement time was calculated as;

$$(G_1\text{-}G_0) / G_0 \times 100\%$$

Where $G_1$ was the tree height or stem diameter at that measurement time, and $G_0$ was the tree height or stem diameter at the previous measurement time. Temperature, relative humidity and rainfall, were recorded at a met-station located approximately 3.3 km away from the study site to compare the climatic conditions of the two separate growing seasons over which tree growth was recorded.

### 3.2.3. Artificial defoliation

Artificial defoliation was used to simulate defoliation by *P. charybdis* and *O. eucalypti*. Both species are chewers, consuming whole leaves with a preference for soft expanding foliage. *Paropsis charybdis* is the most important eucalypt pest in the South Island and usually exhibits a top-down feeding pattern such that outbreaks of the beetle produce what is called “broom top” damage as shoots in the crown top are almost fully defoliated before harder mature foliage is consumed. Each artificial defoliation event was completed over a period of < 2 weeks to minimise difference in initial regrowth between individual trees. Scissors were used to remove whole leaves and buds.

Prior to defoliation, a 50% crown reference point was determined for each tree using an adjusted version of the Colour Digital Image Processing method (Peper and McPherson 2003). Two photos were taken at 3 m distance from each tree, perpendicular to each other. A white cloth background was used for photographing. Total leaf area of the tree crown was estimated using the software Quant Plant Disease Severity v.1.0. The software was also used to estimate the position of a horizontal line such that 50% of the total leaf area was above, and 50% below the line (Figure 3.1a). For the moderate defoliation treatment, all leaves above the 50% reference point were removed (Figure 3.1b). For the severe defoliation treatment, in addition to removing the leaves above the 50% reference point, most of the leaves from the lower crown were removed such that only 10% of the entire crown remained (Figure 3.1c).

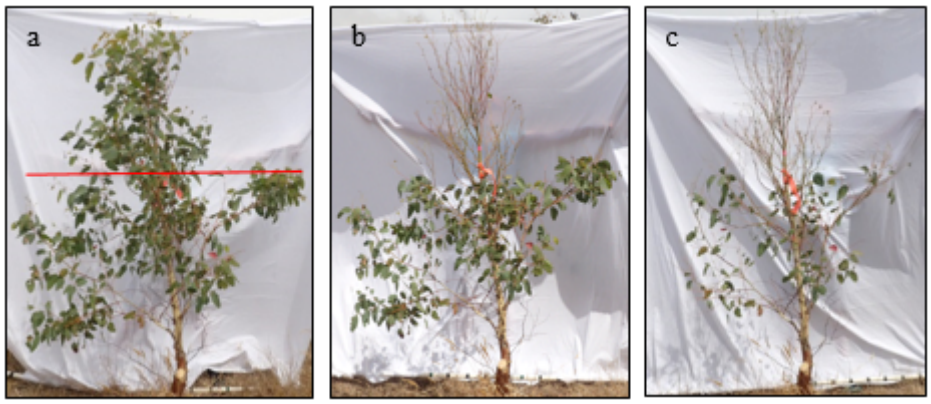



**Figure 3.1** Typical experimental trees showing the different levels of defoliation severity applied: a) undefoliated tree with red solid line separating the crown into the upper and lower 50% leaf area. Moderate defoliation was conducted by removing leaves above the red line; b) moderate defoliation (approximately 50% defoliation of the whole crown); c) severe defoliation (approximately 90% defoliation of the whole crown).

### 3.2.4. Re-foliation estimate

Re-foliation, which refers to the percentage of the defoliated area of the crown which subsequently sprouted new buds and leaves, was visually estimated in January, March, September, October and December 2016. Re-foliation was estimated as a proportion of the defoliated part of each defoliated tree.

### 3.2.5. Insect control

To protect trees from being further defoliated by insects, Confidor (Bayer) with 350g/litre imidacloprid in the form of a suspension concentrate, was applied to all experimental trees. Nine mL Confidor was mixed with 10 L water, and 1 L of this mixture was applied to each tree with an additional 2 L water. Confidor was applied as a soil drench in October 2015 and January 2016 in the first season, but failed to adequately control the defoliators. Thus, manual removal of any defoliators was also conducted each month. The same insecticide was applied in the second season in October 2016 just after a heavy rain event. The control achieved by the insecticide was effective on this occasion because the soil was moist enough to facilitate sufficient uptake of the insecticide. Trees were still checked monthly, and the few insect defoliators found were removed.

### 3.2.6. Data analysis

One-way ANOVA was used to compare mean stem diameter and height between treatments at the start of the experiment. If the ANOVA tests were significant, Tukey Honest Significant Differences (Tukey HSD) was performed to compare multiple pairwise-comparisons between the means of treatments. Analysis of covariance was conducted to test the effect of initial diameter (measured in September 2015) on tree growth using linear regression in R (R Development Core Team 2008), with diameter growth as the response variable and defoliation treatments and initial stem diameter as the explanatory variables. The same analysis was also conducted for testing the effect of initial tree height on tree height growth.

Using the data collected from October 2015 to April 2017 (from week 0 to week 70), R and the function lmer in the lme4 package (Bates et al. 2015) were used to produce linear mixed effect models (LMMs) to analyse the effects of different defoliation treatments on stem diameter and tree height growth. For the defoliation effects on stem diameter growth, the fixed effects part of the model were defoliation treatments and time (with interaction), and the random effects part were random intercepts and slopes with correlation for trees and time and for plot and time. A linear mixed effect model was also used to test the relationship between tree height and defoliation treatments over the same period. The fixed part of the model was the same as the stem diameter model. For the random effects, there were random intercepts and slopes with correlation for trees and time only. The random part of the model indicated that 'tree' made a significant contribution to the variation in tree height. Visual inspection of the residual plots of two models did not reveal obvious deviations from the model assumptions (Figure 3.2). Function glht in R package multcomp (Hothorn et al. 2008) was performed to compare the effect of defoliation treatments on diameter and tree height growth as predicted by the linear mixed effect model mentioned above.

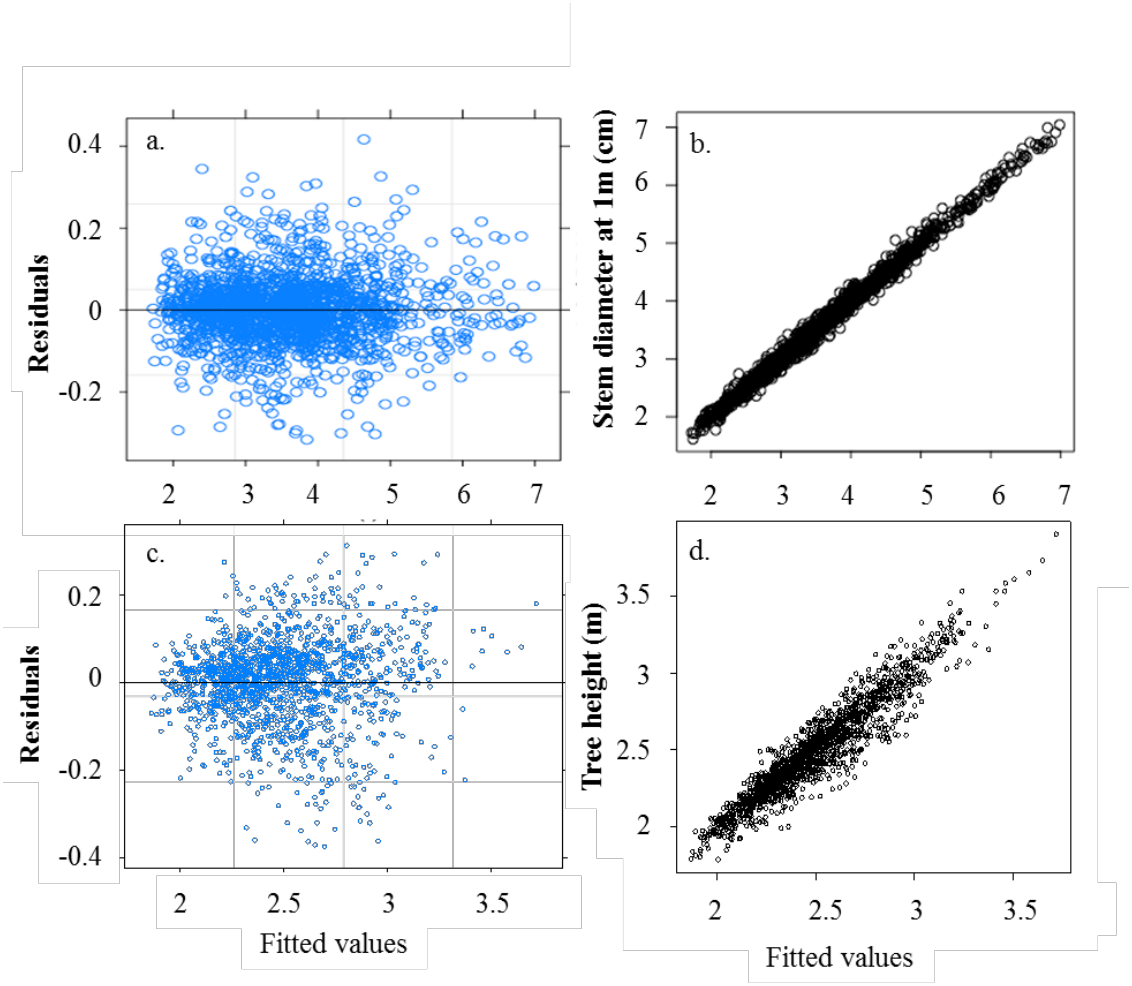



**Figure 3.2** Residual plots (a & c) and fitted vs. actual values plots (b & d) of the linear mixed effect model for stem diameter and height growth.

## 3.3. Results

### 3.3.1. Climate and re-foliation summary

Average daily temperature for both growing seasons (one growing season is from September to April) was 15 ºC. Average daily maximum temperature of the first growing season (2015/16) was 27ºC, 1°C higher than the second (2016/17) season (Figure 3.3). Total rainfall of season 1 (September 2015 to March 2016) and season 2 (September 2016 to March 2017) was 218.6 mm and 236 mm respectively, and the total rainfall during the intervening winter (April 2016 to August 2016) was 181.6 mm.

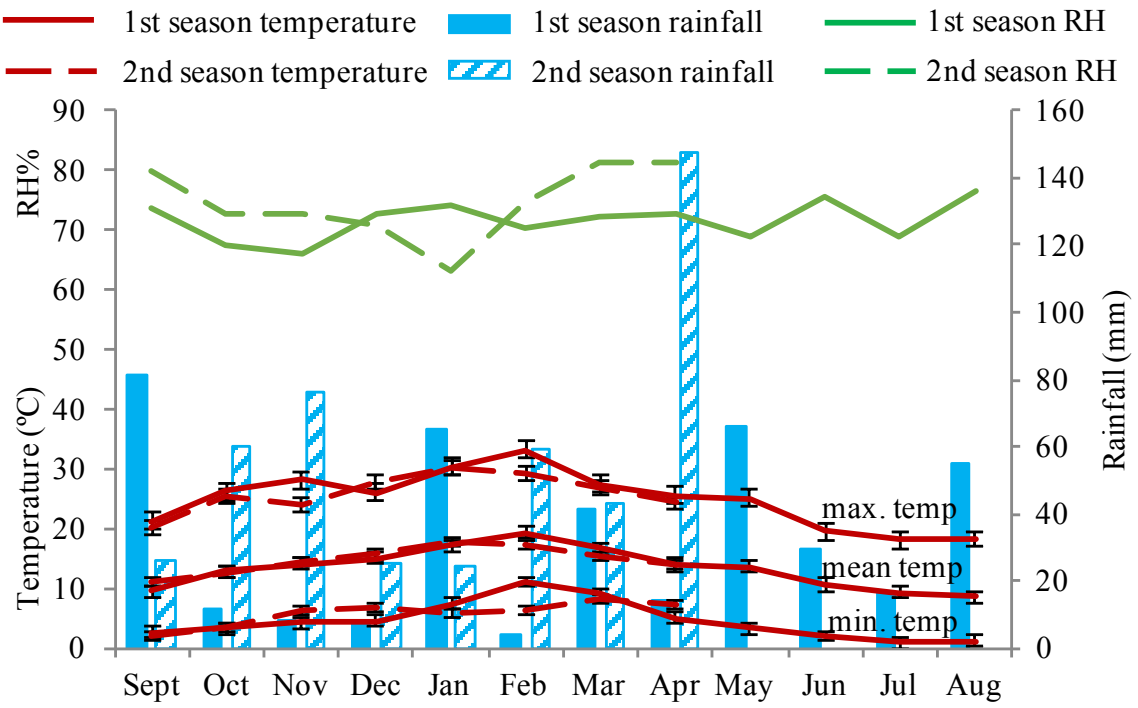



**Figure 3.3** Average daily maximum, mean and minimum temperature per month, average daily relative humidity (RH) and total monthly rainfall during two growing seasons.

All the trees survived defoliation over the experimental period. Twelve weeks (January 2016) after the spring defoliation, trees subject to the severe treatment had more foliar regrowth (the percentage of the defoliated area of crown with new buds and leaves) than those subject to the moderate treatment with average percentage of re-foliation of 59% and 68% for Sp50 and Sp90 respectively. These values increased to 74% and 84% average re-foliation in week 20 (Table 3.3). However, there were large variations in the amount of re-foliation within treatments (Figure 3.4). Severe treatments, Sp90 and SpLs90, showed smaller variation in re-foliation than the moderate treatments, Sp50 and SpLs50. Trees were observed to have two regrowth periods (in spring and during summer) during each growing season, and the difference between re-foliation percentage of moderate and severe treatments, and variation of each treatment became smaller after the second regrowth period. Trees subject to late summer moderate and severe treatments, Ls50 and Ls90, showed average re-foliation of 20% and 44% in week 46, approximately 6 months after the late summer defoliation event. Re-foliation of Ls90 was faster

than Ls50 until week 58, at which time most trees in both treatments had fully re-foliated and looked like normal undefoliated trees. Trees in the spring plus late summer severe defoliation treatment (SpLs90) only had 18% re-foliation at week 46, compared to trees that were severely defoliated only in late summer without a spring defoliation which re-foliated by an average of 44% in the same period. However, the re-foliation of SpLs90 caught up with other treatments in week 50 (Figure 3.4). Average re-foliation of SpLs50 and SpLs90, which had been defoliated in both spring and late summer, was lower than Ls50 and Ls90 which were only defoliated once in late summer (week 58). Variation in re-foliation was also higher in moderate treatments than severe treatments by the second season, but variation declined as trees grew.

In February/March 2016, one tree in the Sp50 treatment, three trees in the SpLs50 and one tree in the SpLs90 had obvious bud damage caused by *S. macropetana*, which limited foliar regrowth on these trees. However, since these trees recovered quickly in the second season and the foliage regrowth had caught up with that of other trees in December 2016 (more than 50% of previous defoliated area had re-sprouted), they are still considered in the following analysis.

**Table 3.3** Average percentage of artificially defoliated area of crown showing re-foliation from 12-58 weeks after defoliation events. Sp50 and Sp90 were defoliated in week 0 while Ls50 and Ls90 were defoliated in week 22. SpLs50 and SpLs90 were defoliated in both week 0 and week 22. (Sp – spring defoliation event; Ls – late summer defoliation event).

| Treatments | Oct-15 (week 0) | Jan-16 (week 12) | Mar-16 (week 20) | Mar-16 (week 22) | Sep-16 (week 46) | Oct-16 (week 50) | Dec-16 (week 58) |
|---|---|---|---|---|---|---|---|
| Sp50 | Sp | 59% | 74% | | - | - | - |
| Sp90 | Sp | 68% | 84% | | - | - | - |
| Ls50 | | - | - | Ls | 20% | 54% | 74% |
| Ls90 | | - | - | Ls | 44% | 65% | 73% |
| SpLs50 | Sp | 56% | 72% | Ls | 20% | 47% | 66% |
| SpLs90 | Sp | 71% | 81% | Ls | 18% | 63% | 63% |

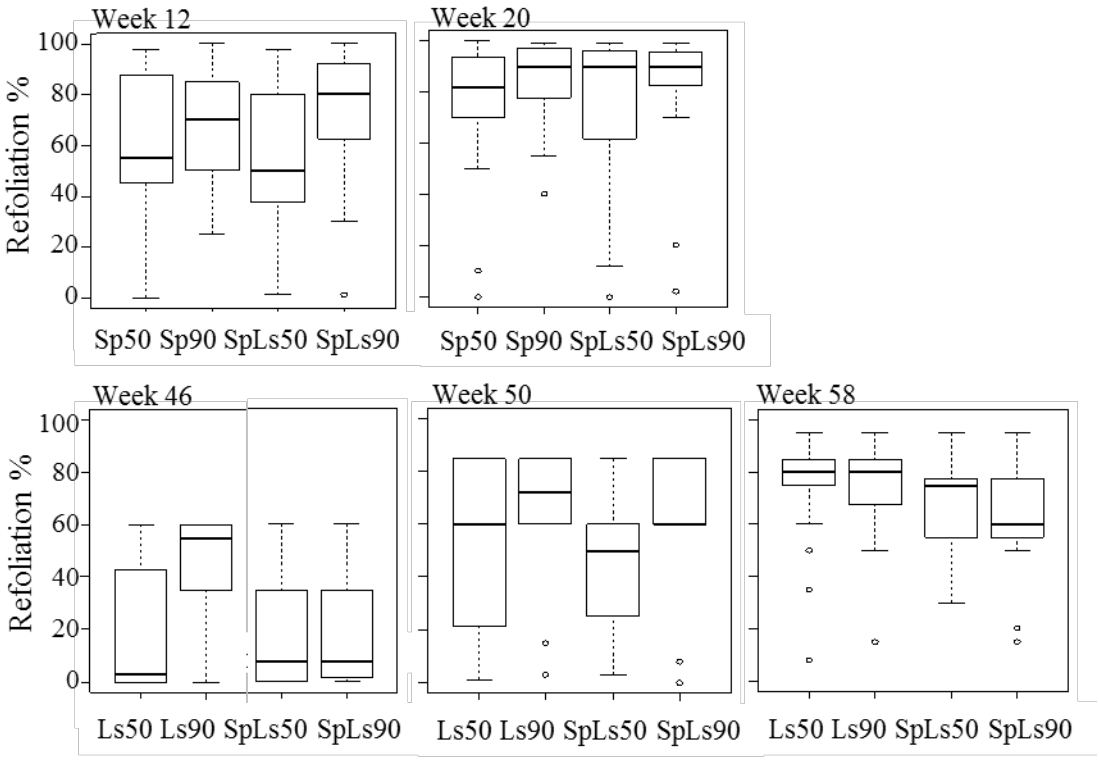



**Figure 3.4** Boxplots showing variation in percentage of defoliated shoots that had re-foliated at 12 to 58 weeks after the first spring defoliation event (Week 0) for trees under each of 6 treatments.

### 3.3.2. Defoliation impact on stem diameter growth

Mean stem diameter (at 1 m) of different treatments at the start of the experiment (measured just after spring defoliation) varied from 2.84 to 3.4 cm, but were not significantly different from each other (Table 3.4, Figure 3.5a). Trees in all treatments increased in stem diameter by an average of 20% to 54% over the 70 week experiment period. Control trees that were not subject to defoliation exhibited the greatest overall growth, with a 54% increase in mean diameter growth compared to diameter in week 0 (Table 3.4). In contrast, trees subject to a single moderate defoliation had a mean stem diameter increase of 49% and 41% for spring (Sp50) and late summer (Ls50) treatments respectively, while those subject to a single severe defoliation only increased by 36% (Sp90) and 33% (Ls90) by week 70. Trees defoliated both in spring and late summer, showed the least increase in stem diameter (21% and 20% for SpLs50 and SpLs90 respectively).

In the first growing season, a reduction in growth increment was observed for defoliated compared to undefoliated trees from December 2015 to February 2016. During the first growing season, late summer treatments (Ls50 and Ls90) had similar increases in diameter as control trees between most of the consecutive measurements because they were effectively control trees themselves – they had not yet been defoliated (Figure 3.5b, Table 3.4). After spring defoliation in October 2015, Sp50 showed less diameter growth relative to control trees but had more diameter increase than Sp90. At the end of the season, , Sp50 showed 4% less diameter growth, and Sp90 showed 10% less growth, relative to control trees. SpLs90 had similar diameter increase to Sp90 because they were subject to the same treatment at this point.

Surprisingly, diameter growth of SpLs50 was just as low as SpLs90 (average 8%) during season 1, at less than half that of the control trees.

In winter (April to September), control trees grew by an average 11% in diameter, while Sp50 and Sp90 gained 2% and 5% less respectively (Figure 3.5b). Not surprisingly, trees subject to severe late summer defoliation grew less than those subject to the moderate treatment, with only a 5% increase in stem diameter for Ls50 and only 3% for Ls90. Spring plus late summer defoliation resulted in growth increase similar to Ls90 (3%).

Over season 2, increases in the diameters of Sp50 and Sp90 trees were comparable to control trees (Figure 3.5b, Table 3.4). Trees subject to late summer moderate defoliation (Ls50) grew more than trees subject to late summer severe defoliation (Ls90) by 5%, while trees subject to spring plus late summer defoliation (SpLs50) had similar increases in diameter to trees subject to severe defoliation (SpLs90). Growth rate of Ls50 trees decreased after defoliation, but increased to be comparable to the control trees by January 2017 (Figure 3.5b). SpLs50 and SpLs90 had the lowest increase in diameter over the second season (21% and 20%).

Analysis of covariance on the effect of initial stem diameter on diameter growth showed that the effect of initial stem diameter was not significant. The LMM on the relationship of stem diameter and defoliation treatments showed that the estimated stem diameter in week 0 (the intercepts for treatments) for samples in Control, Sp50, Sp90, Ls50, Ls90, SpLs50 and SpLs90 were 3.14±0.26 cm, 2.98±0.27 cm, 2.78±0.27 cm, 3.03±0.19, 3.52±0.27 cm, 3.25±0.27 cm and 3.19±0.27 cm, indicating that there was no significant difference in stem size at the start of the experiment. However, the interaction between time and treatment (the slopes) was significant, indicating that defoliation treatments had significant effects on stem diameter and there was significant difference in growth rate between at least two of the treatments. The highest estimated weekly growth rate (the slope) was for the control treatment (0.021±0.002 cm/week), followed by Sp50 (0.016 ± 0.002 cm/week) (Figure 3.6). The spring plus late summer defoliation treatments (SpLs50 and SpLs90) had the lowest estimated slopes (0.008 and 0.006 cm/week), and therefore the slowest growth rates. The random effects part of the model indicated that plot and tree (micro-environment) made significant contributions to the variation in diameter growth.

### *Effect of defoliation severity on diameter growth*

For the spring defoliation treatment, only severe defoliation significantly reduced diameter growth ($P<0.01$) compared to the control (Figure 3.6). Although diameter growth rate of Sp50 and control were not significantly different, Sp50 gained 9% less diameter growth than control trees over the experiment period. For late summer defoliation and spring plus late summer defoliation treatments, estimated growth rates were significantly different from control ($P<0.001$). Slopes for late summer treatments were 0.13 cm/week and 0.11 cm/week for moderate and severe treatment, and only 0.008 cm/w and 0.006 cm/week for SpLs50 and SpLs90. There was no significant difference between moderate and severe defoliation treatments for spring or late summer defoliation, although estimated growth rates decreased with increased defoliation severity.

**Table 3.4** Mean stem diameter at 1 m as measured in October 2015, March 2016 (end of the 1st season), September 2016 (start of the 2nd season) and April 2017 (end of the 2nd season), which were 0, 20, 46 and 70 weeks after spring defoliation. Percentage increase is shown for stem diameter over season 1, winter, season 2 and the entire 70 week period. Note: Ls50 and Ls90 were effectively control trees for season 1 as they were not defoliated until the end of that season.

| Treat-ments | Mean stem diameter ± SE | | | | Mean diameter increase in season 1 | Mean diameter increase in winter | Mean diameter increase in season 2 | Mean diameter increase whole period |
|---|---|---|---|---|---|---|---|---|
| | Oct-15 | Mar-16 | Sept-16 | Apr-17 | | | | |
| Control | 3.09±0.16 | 3.59±0.17 | 3.98±0.18 | 4.69±0.21 | 17% | 11% | 18% | 54% |
| Sp50 | 3.01±0.18 | 3.36±0.17 | 3.64±0.17 | 4.31±0.19 | 13% | 9% | 19% | 49% |
| Sp90 | 2.84±0.19 | 3.02±0.19 | 3.21±0.201 | 3.79±0.22 | 7% | 6% | 19% | 36% |
| Ls50 | 2.93±0.13 | 3.44±0.13 | 3.61±0.14 | 4.10±0.16 | 18% | 5% | 14% | 41% |
| Ls90 | 3.40±0.24 | 3.95±0.24 | 4.07±0.241 | 4.42±0.25 | 18% | 3% | 9% | 33% |
| SpLs50 | 3.20±0.22 | 3.44±0.23 | 3.54±0.24 | 3.88±0.26 | 8% | 3% | 9% | 21% |
| SpLs90 | 3.21±0.22 | 3.45±0.23 | 3.55±0.23 | 3.82±0.25 | 8% | 3% | 8% | 20% |

***Effect of repeated defoliation on diameter growth***

Trees subject to spring plus summer defoliation events gained less than half the growth in stem diameter relative to the control (Table 3.4). Trees subject to moderate defoliation in both spring and late summer (SpLs50) had significantly lower estimated diameter growth rate than trees that were moderately defoliated in either only spring or only late summer (single event) (both $P<0.001$). The estimated growth rate of SpLs50 was 0.008 cm/week which was only half of that of Sp50. Average growth gain at the end of the second season of SpLs50 was 22% and 19% less than Sp50 and Ls50. However, estimated growth rate of SpLs90 had no significant difference from Sp90 ($P=0.0928$) and Ls90. Growth increase of SpLs90 was 16% and 13% less than Sp90 and Ls90.

***Effect of defoliation timing on diameter growth***

Trees exposed to spring defoliation gain 3% - 4% more in stem diameter increase than those subject to late summer defoliation with the same defoliation severity over the experiment period (Table 3.5). However, there was no significant difference between estimated growth rates of Sp50 and Ls50, and between Sp90 and Ls90 (Figure 3.6).

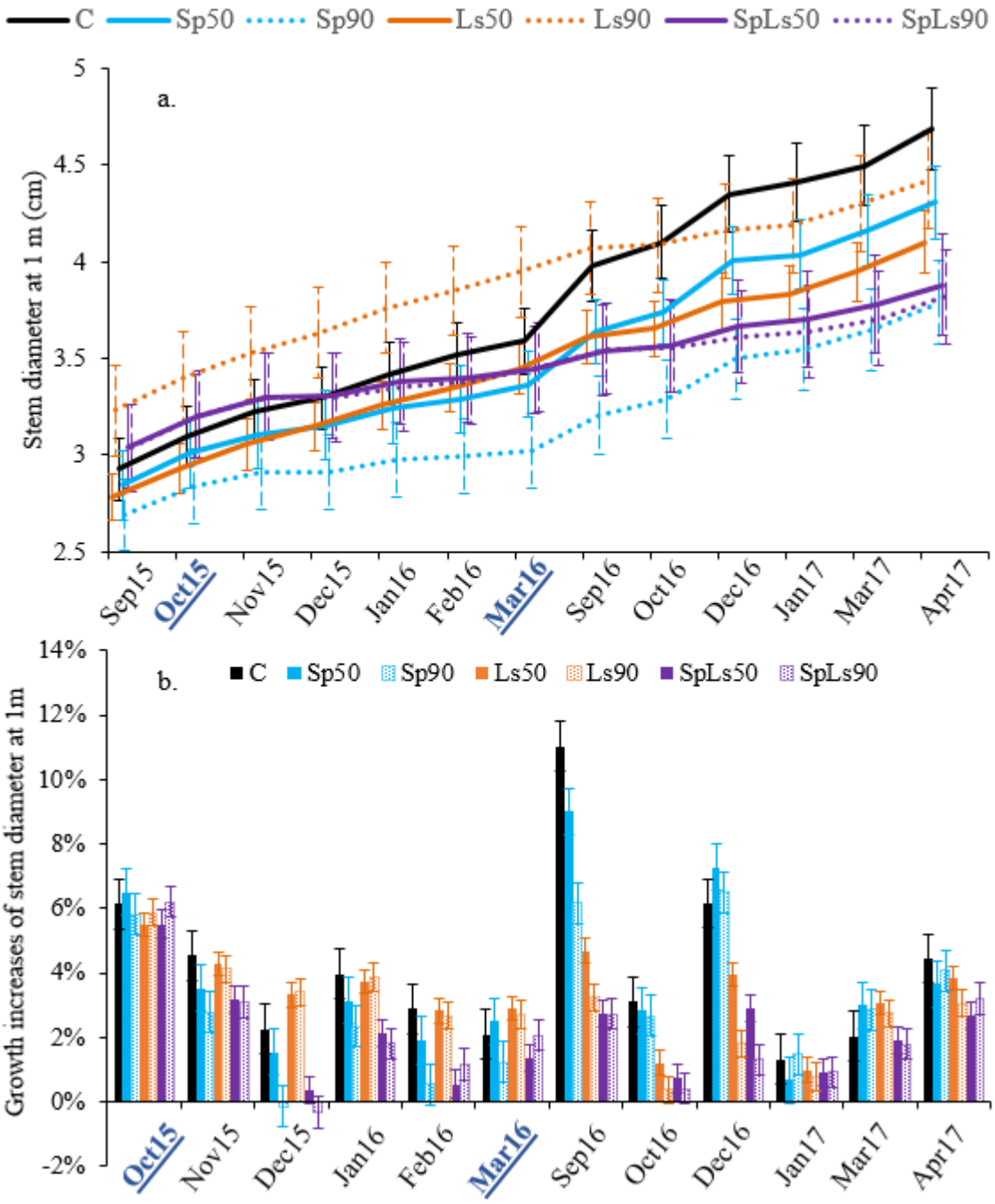
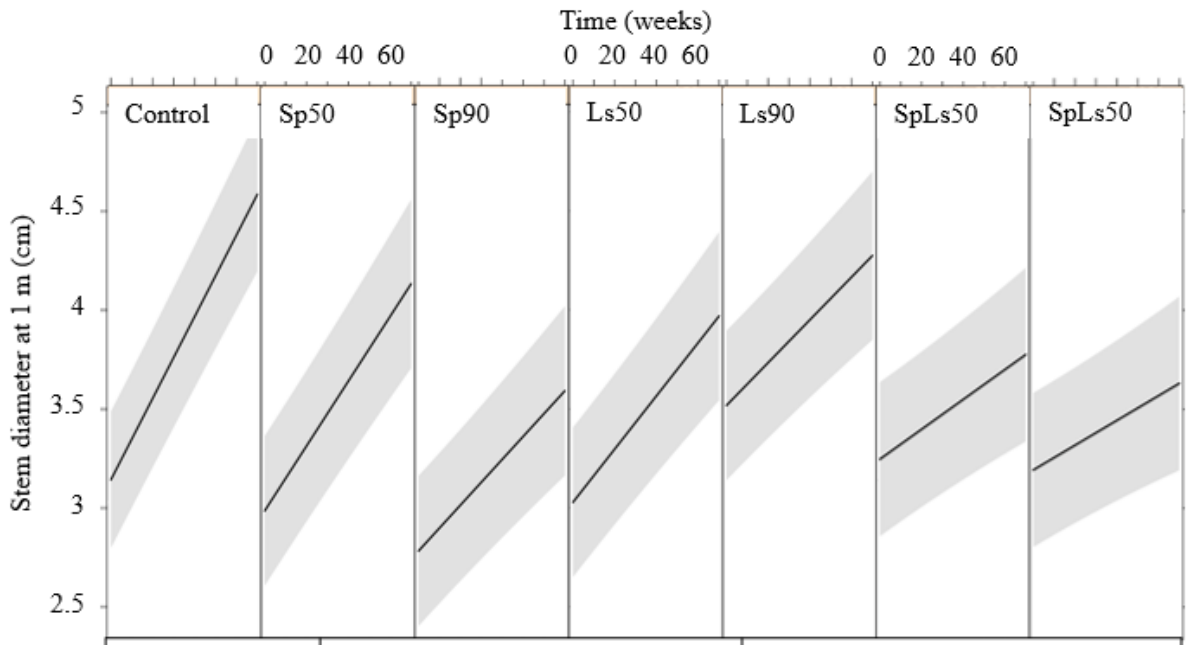



**Figure 3.5** a.) Mean (± SE) stem diameter (at 1 m) of trees subject to different defoliation treatments from September 2015 (4 weeks before spring defoliation) to April 2017 (70 weeks after spring defoliation); b) Mean (± SE) stem diameter (at 1 m) growth increases on each measurements occasion from October 2015 to April 2017 (growth increase of Oct15 was the ratio of increase in stem diameter in October 2015 to stem diameter in September 2015, other growth increases were the ratio of increase in stem diameter to stem diameter in previous measurement). Months in which defoliation was conducted are shown in blue bold font with underline: Sp = October, Ls – March.




| Treatments | Intercepts (cm) (stem diameter at week 0) | Slopes (cm/week) (growth rates) |
|---|---|---|
| Control | 3.141 ± 0.255 | 0.021 ± 0.002 a |
| Sp50 | 2.984 ± 0.268 | 0.016 ± 0.002 ab |
| Ls50 | 3.028 ± 0.193 | 0.013 ± 0.002 b |
| Sp90 | 2.781 ± 0.268 | 0.012 ± 0.002 bc |
| Ls90 | 3.517 ± 0.267 | 0.011 ± 0.002 bc |
| SpLs50 | 3.247 ± 0.271 | 0.008 ± 0.002 c |
| SpLs90 | 3.193 ± 0.273 | 0.006 ± 0.002 c |

**Figure 3.6** Stem diameter for each of the different treatments predicted by the fixed part of the linear mixed effects model for stem diameter; table showing the intercepts (±SE) and slopes (±SE) of different treatments estimated by the model; comparisons of slopes of treatments tested by glht function in Multcomp package in R are shown by lowercase letters (treatments shared any common letter were not significantly different).

### 3.3.3. Defoliation impact on tree height growth

Mean tree heights of trees at week 0 (October 2015) were not significantly different between treatments. Average tree height across all treatments was 2.23 ± 0.41 m. Control trees showed the largest mean height increase (36%) followed by Sp50, with 4% less (Table 3.5). Unlike diameter growth, which was greater for Ls50 than Sp90, both of these treatments showed similar height growth at 28% and 27% at the end of the two growing seasons. SpLs90 had the least height increase over the experiment period (20%). The variations in growth between treatments were smaller for tree height than stem diameter.

Over the first growing season, Sp50 trees gained the most height growth, 1% and 2% more than control and Ls50 (which was not subject to defoliation until the end of season 1). Ls90, which was not defoliated until late summer, showed a slower increase in height (10%) than control trees, but exhibited a similar increase as trees subject to spring defoliation (Sp90, SpLs50 and SpLs90). All treatments had minimal height growth during winter.

In the second growing season, differences in growth increase per sampling period were observed between treatments by January 2017 (Figure 3.7b). Control trees had the greatest increase in height growth especially in January 2017, and had the most height growth increase over season 2 (22%). Sp90 trees grew 3% less in height compared to Sp50 in season 1, but both showed similar height increase in season 2 (16%). By the end of the experimental period Sp90 trees were still shorter than Sp50 trees on average (Figure 3.7a). Late summer defoliation suppressed height growth, with trees suffering moderate and severe defoliation at this time increasing in height by only 14% and 12% respectively. Trees of Ls90 and trees defoliated in both spring and summer, regardless of severity, had the slowest height increase.

Result of the analysis of covariance on the effects of initial tree height on tree height growth showed that the effect of initial tree height was not significant. The LMM on tree height showed that the estimated tree height at week 0 (the intercepts for treatments) was not significantly different between treatments. However, the interaction between time and treatment (the slopes) was significant, indicating defoliation treatments had significant effects on tree height and there were significant differences in height growth rates between at least two treatments. Slopes, which represent growth rate of tree height, were largest for control at 0.01 m/week followed by Sp50 at 0.008 m/week. Trees subject to both spring and late summer defoliation (SpLs50 & SpLs90) and subject to severe late summer defoliation (Ls90) had the smallest estimated growth rate at 0.006 m/week. Trees defoliated in spring regardless of defoliation severity and trees moderately defoliated in late summer had the same estimated growth rate (Figure 3.8).

**Table 3.5** Mean tree height as measured in October 2015, March 2016 (end of the 1st season), September 2016 (start of the 2nd season) and April 2017 (end of the 2nd season), which were 0, 20, 46 and 70 weeks after spring defoliation. Percentage increase in tree height is shown for season1, winter, season 2 and over the entire monitoring period. Note: Ls50 and Ls90 were effectively control trees for season 1 as they were not defoliated until the end of that season.

| Treat-ments | Mean stem diameter ± SE | | | | Mean height increase in season 1 | Mean height increase in winter | Mean height increase in season 2 | Mean height increase whole period |
|---|---|---|---|---|---|---|---|---|
| | Oct-15 | Mar-16 | Sep-16 | Apr-17 | | | | |
| Control | 2.23±0.04 | 2.50±0.06 | 2.50±0.06 | 3.04±0.07 | 12% | 0% | 22% | 36% |
| Sp50 | 2.19±0.04 | 2.47±0.04 | 2.48±0.04 | 2.88±0.06 | 13% | 0% | 16% | 32% |
| Sp90 | 2.16±0.04 | 2.38±0.05 | 2.37±0.05 | 2.76±0.08 | 10% | 0% | 16% | 28% |
| Ls50 | 2.25±0.05 | 2.52±0.06 | 2.51±0.06 | 2.85±0.08 | 12% | 0% | 14% | 27% |
| Ls90 | 2.33±0.05 | 2.55±0.05 | 2.55±0.05 | 2.86±0.06 | 10% | 0% | 12% | 23% |
| SpLs50 | 2.21±0.03 | 2.44±0.06 | 2.44±0.06 | 2.72±0.07 | 10% | 0% | 12% | 23% |
| SpLs90 | 2.23±0.04 | 2.46±0.07 | 2.46±0.07 | 2.69±0.07 | 10% | 0% | 10% | 20% |

***Effect of defoliation severity on tree height growth***

Trees that were not defoliated (Control) had the fastest estimated growth rate at 0.01 m/week, which was 0.002 m/week and 0.003 m/week faster than Sp50 and Sp90 (Figure 3.8). The impact of spring defoliation on height growth was less than on diameter growth. Moderate defoliation in spring (Sp50) did not significantly affect height growth while severe spring

defoliation (Sp90) had a marginally non-significant (P=0.0847) effect. Slopes of moderated and severe spring defoliation were not significantly different. Moderate late summer defoliation resulted in a marginally non-significantly reduction in height growth relative to control (P=0.064), but it significantly reduced diameter growth. Severe late summer defoliation significantly reduced height growth relative to controls (P=0.006), but was not significantly different from moderate defoliation. Both spring plus late summer defoliation treatments (SpLs50 and SpLs90) significantly reduced height growth compared to Control (P<0.01) and had the same estimated height growth rate.

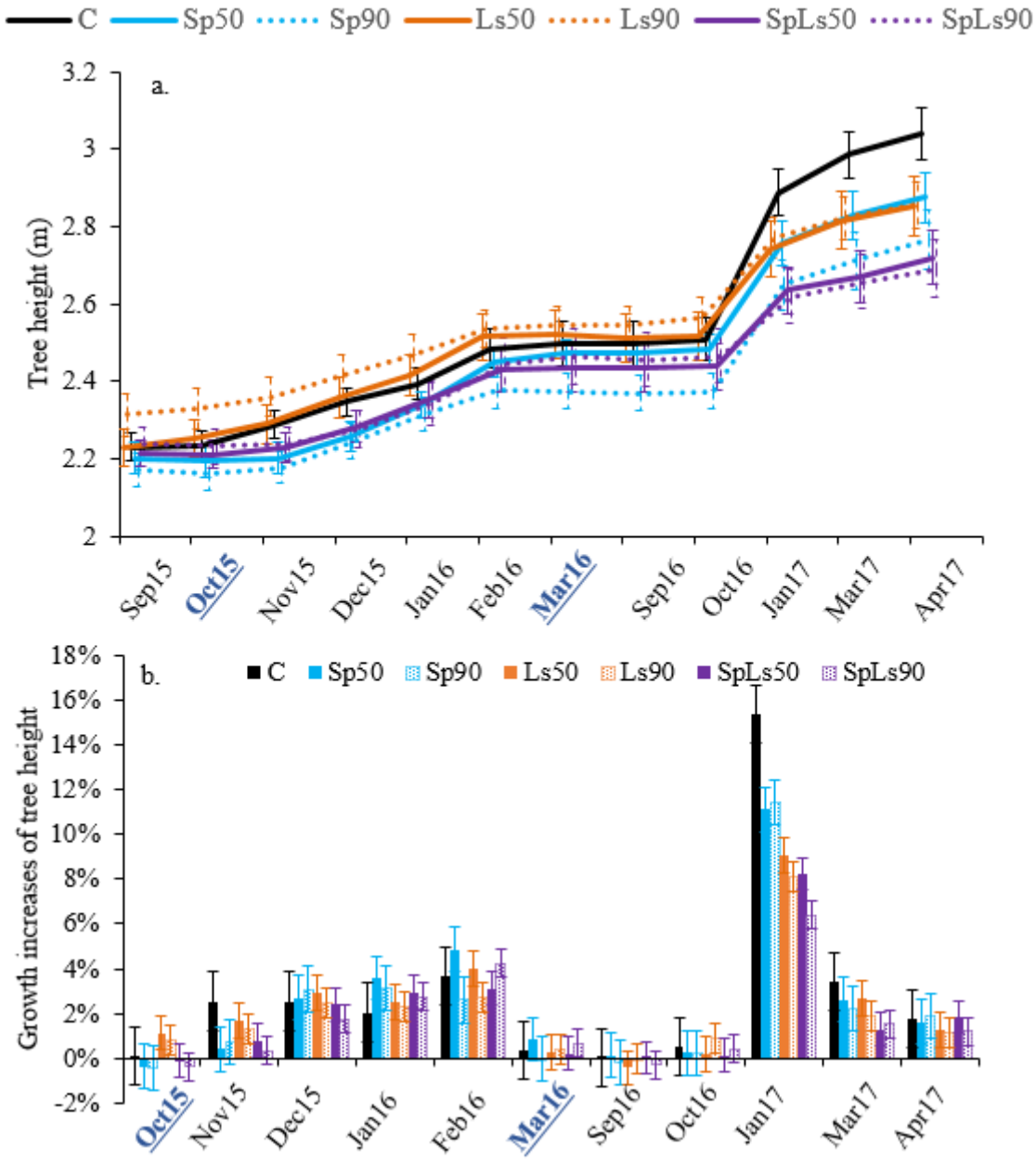
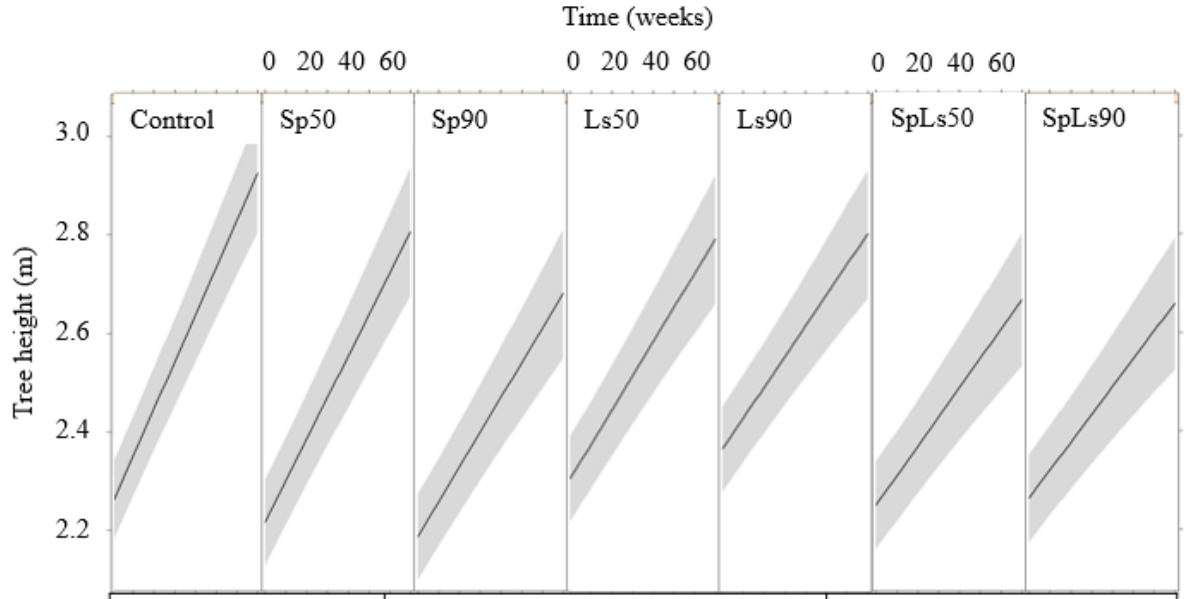



**Figure 3.7** a) Mean (± SE) tree height of trees subject to 7 defoliation treatments from September 2015 (4 weeks before spring defoliation) to April 2017 (70 weeks after spring defoliation); b) Mean (± SE) tree height growth increases from October 2015 to April 2017 (growth increase of Oct15 was the ratio of increase in height in October 2015 to height in September 2015, other growth increases were the ratio of increase in height to height in previous measurement). Months in which defoliation events occurred are shown in blue bold font with underline: Sp = October, Ls – March.




| Treatments | Intercepts (m) (tree height at week 0) | Slopes (m/week) (growth rates) |
|---|---|---|
| Control | 2.252 ± 0.060 | 0.010 ± 0.001 a |
| Sp50 | 2.214 ± 0.063 | 0.008 ± 0.001 ab |
| Sp90 | 2.184 ± 0.063 | 0.007 ± 0.001 ab |
| Ls50 | 2.303 ± 0.044 | 0.007 ± 0.001 ab |
| Ls90 | 2.364 ± 0.063 | 0.006 ± 0.001 b |
| SpLs50 | 2.248 ± 0.064 | 0.006 ± 0.001 b |
| SpLs90 | 2.263 ± 0.064 | 0.006 ± 0.001 b |

**Figure 3.8** Tree height for each of the different treatments predicted by the fixed part of the linear mixed effects model for tree height; table showing the intercepts (±SE) and slopes (±SE) of different treatments estimated by the model; comparisons of slopes of treatments tested by glht function in Multcomp package in R are shown by lowercase letters (treatments shared any common letter were not significantly different).

### *Effect of defoliation repeated defoliation on tree height growth*

Estimated slopes for Sp50, Ls50 and SpLs50 are 0.008, 0.007 and 0.006 m/week, and there was no significant difference between these treatments (Figure 3.8). SpLs90 and Ls90 had very similar slopes at 0.006 m/week, and there was no significant difference among Sp90, Ls90 and SpLs90. Both moderate and severe spring plus late summer defoliation treatments significantly reduced height growth relative to control, while single defoliations (either spring or late summer defoliation) did not significantly reduce height growth.

### *Effect of defoliation timing on height growth*

There was no significant difference in estimated height growth rates between Sp50 and Ls50, and between Sp90 and Ls90 (Figure 3.8). Trees under all the treatments showed similar height growth in winter, in contrast with diameter growth. Trees subject to spring defoliation showed

greater growth in the following season than those subject to late summer defoliation. Also, severe late summer defoliation significantly reduced height growth while severe spring defoliation did not.

## 3.4. Discussion

### 3.4.1. Key results

1. All trees survived and increased in diameter and height over the experiment period. Artificial defoliation had a larger impact on *E. bosistoana* stem diameter growth than on tree height over the experiment period.

2. There were no significant differences in stem diameter and tree height between treatments at the start of the experiment. After two growing seasons, defoliated trees exhibited lower average growth increases in stem diameter and tree height. Only spring moderate defoliation did not significantly reduce tree growth, while other defoliation treatments significantly reduced either diameter or height growth.

3. Severity of defoliation had a negative relationship with both tree diameter and height growth rates, but there was no significant difference observed between moderate and severe defoliation treatments for spring, late summer or spring plus late summer.

4. Defoliation timing had a critical impact on growth. Late summer defoliation had a larger impact than spring defoliation, and this was exacerbated by defoliation severity.

5. Spring plus late summer defoliation events had the greatest negative impact on stem diameter and tree height growth relative to defoliation treatments. Repeated defoliation reduced average diameter growth by 16 and 28% relative to a single severe or moderate spring defoliation event. However, the difference was only significant for the moderate severity.

### 3.4.2. Impacts of defoliation on *E. bosistoana* growth

#### *Survival after defoliation*

All trees survived during the experiment period. This is consistent with previous artificial defoliation studies. In studies using artificial defoliation methods, *E. globulus* (Collett and Neumann 2002), *E. grandis* (De Oliveira et al. 2014), *E. marginata* (Wills et al. 2004), *E. regnans* (Candy et al. 1992) and *E. nitens* (Elek 1997, Candy 2000b, Elek and Baker 2017), have been shown to survive at least one complete defoliation event, although significant mortality was reported for *E. regnans* in Australia when completely defoliated for two consistent years in late summer (March) (Candy et al. 1992). *Eucalyptus marginata* survived 13 consecutive years of complete defoliation, but their growth stopped within 3 years (Wills et al. 2004).

***Tolerance of single defoliation events***

The finding that moderate (about 50%) spring defoliation did not significantly reduce either diameter or height growth increment, agrees with some previous artificial defoliation studies on other *Eucalyptus* species (e.g. Candy et al. 1992, Elek 1997, Collett and Neumann 2002, Pinkard et al. 2011b, Barry et al. 2012, Quentin et al. 2012, De Oliveira et al. 2014). However, the threshold level of defoliation above which growth increment will be affected differs between species and studies. The difference is likely due to factors including different host species, defoliation agents, patterns of defoliation (part of crown defoliated and foliage age), site factors (e.g. climate factors) and measurement point on the stem (Kulman 1971). Some studies on the widely planted *E. globulus*, *E. grandis*, *E. nitens* and *E. regnans*, have shown *Eucalyptus* are able to compensate for moderate (40-60%) defoliation (e.g. Eyles et al. 2009, Rapley et al. 2009, Barry et al. 2012). However, there are conflicting results for some species. Half of the studies on *E. globulus*, which is the most studied *Eucalyptus* species for defoliation response (Appendix 1), have shown that ~ 50% defoliation (usually 50% of the crown length) did not have a significant impact on stem diameter and height growth (Collett and Neumann 2002, Eyles et al. 2009, Pinkard et al. 2011b, Barry et al. 2012, Quentin et al. 2012), but all of these studies used seedlings with the exception of Collett and Neumann (2002) who used 1.7 years old trees. Barry et al. (2012) stated that foliar carbohydrates, which were correlated to growth recovery after defoliation, were highest in seedlings, so they achieve greater growth after defoliation than larger trees. In contrast, other studies showed that around 50% defoliation reduced either diameter or both diameter and height growth. In two of these studies, seedlings were planted in pots (Quentin et al. 2010, Barry and Pinkard 2013) while the other studies mentioned above were conducted as field trials This may affect tree growth responses because of different nutrition and space availability. One study that found 45% defoliation significantly reduced stem diameter growth was conducted on 4 years old *E. globulus*, which were much larger than seedlings used in most of other studies (Quentin et al. 2011). This may imply that growth response following defoliation can be affected by the age of *Eucalyptus.* Controlled experiments are required to better understand the relationship between tree age and recovery ability from defoliation.

Defoliation timing is also a possible driver of the differences observed among these studies. Late summer defoliation or winter defoliation tends to cause significant reductions in growth (Pinkard et al. 2006b, Quentin et al. 2011) while spring defoliation tends to produce more variable results. Reduction in tree growth is generally higher when defoliation is caused by natural agents (e.g. insects) rather than artificial clipping, possibly because the durations of defoliation events are longer than artificial defoliation which usually being done within hours depending on tree size of individual tree.

Many artificial defoliation studies estimate defoliation as a percentage of the crown length, but some studies assess defoliation as a percentage of current season adult foliage, making comparisons difficult. Studies of natural defoliation and managed insect defoliations tend to use visual assessments, especially the Crown Damage Index (Stone and Coops 2004). There is consistent evidence that removing upper crown foliage has a greater impact on tree growth than removing lower crown foliage (Collett and Neumann 2002, Pinkard et al. 2006b, Pinkard et al. 2007a). Kulman (1971) noted that as foliage age increases, the importance of that foliage to tree growth decreases, which may explain why removal of upper crown foliage, which usually consists of more new leaves, has greater influence on tree growth than removal of lower crown

foliage. The position on the stem where diameter growth is measured can also differ between studies because seedlings are always measured at the base of the stem, but larger trees are measured at breast height. This could make comparisons between studies even more difficult as Kulman (1971) claimed the impact of defoliation on stem diameter growth could vary at different heights along the stem.

A conflicting result in this study was that the increase in stem diameter of SpLs50 trees was just as small as that of SpLs90 trees at the end of the first season. This is despite the fact that Sp90 diameter growth was less that Sp50 diameter growth over the same period and these two treatments were effectively the same as SpLs50 and SpLs90 as late season defoliation had not yet occurred. This lack of difference between SpLs50 and SpLs90 is possibly because there were 3 trees in SpLs50 treatment with relatively larger neighbouring trees which may have increased competition for light and nutrition. There was also a lot of *S. macropetana* damage on buds (about 40% of new buds which re-sprouted following artificial spring defoliation were damaged by *S. macropetana*) on three trees within SpLs50 during the period leading up to the end of season 1 measurement. *Strepsicrates macropetana* damage also affected the trees in Ls90 treatment close to the end of season 1, leading those trees to having only a10% height growth increased in that season, similar to defoliated trees.

***Tolerance for repeated defoliation events***

Trees subject to spring plus late summer defoliation in the same season had the least growth increase in both stem diameter and tree height over the experiment period, and also had significantly lower estimated growth rates than control trees from statistical analysis. Repeated defoliation had a greater impact on tree growth than a single defoliation event, which is consistent with previous studies. Defoliation impact is typically greater if defoliation is frequent and severe (Barry et al. 2012, Pinkard et al. 2017). Some forestry insect pest outbreaks can last for several years. *Choristoneura occidentalis* Freeman (Alfaro et al. 2014) and *Sirex noctilio* Fabricius (Hurley et al. 2007), have been found to have devastating impacts on the economic and ecological value of forests during outbreaks of 7-11 years (in Canada) and 4-6 years (in New Zealand and Australia) respectively. Foliage wounded by insects also appears to attract more future attack than unwounded foliage due to the release of specific volatiles in response to insect feeding (Kendrick and Raffa 2006). This implies that trees subject to defoliation caused by insect outbreaks in spring, are prone to suffer from more insect attack later in the season. As such, more efforts should be placed on insect pest monitoring to prevent a second outbreak even if the first outbreak event only causes damage under the threshold that cannot cause significant reductions in tree growth. Moreover, weakened defoliated trees may allow a build-up of secondary insect pests (usually bark beetles and borers) and fungi which require physiological weakened trees to boost their population (Kulman 1971). Consequently, pests and post-defoliation climate conditions should be closely monitored if moderate defoliation takes place early in the season.

Trees in the spring plus late summer severe defoliation treatment (SpLs90) had much fewer re-foliation at week 46 compared to trees that were severely defoliated only in late summer without a spring defoliation, which re-foliated by an average of 44% in the same period. This indicates that the negative impact of late summer defoliation is compounded if preceded by spring defoliation.

***Impact of defoliation timing***

In the current study, trees subject to late summer defoliation gained less diameter growth in winter than trees subject to spring defoliation which experienced a typically dry growing season before the onset of winter. This is likely because most defoliated branches of trees in the spring defoliation treatments re-foliated before winter, but those defoliated in late summer did not have a chance to re-foliate. This allowed the spring moderately defoliated trees gain similar growth as control trees. Moderate defoliation only significantly reduced diameter growth when defoliation was conducted in late summer, which also indicates that late summer defoliation has greater impact on diameter growth than spring defoliation. Candy et al. (1992) studied *E. regnans* in Australia finding that only complete late season defoliation for 2 consecutive years caused significant mortality. Greater impact of late season defoliation may be explained by two reasons: one is that new leaves and twigs remain unlignified after late season defoliation and suffer more easily from winter damage (Kulman 1971). The other possible cause is that late season defoliation lowers photosynthesis in winter reducing the energy stock for the next growing season. In woody plants, stored carbohydrates play a vital role in growth (Kozlowski 1992), and it has been shown that starch pools are highest in leaves of *E. globulus* relative to other part of the tree (Eyles et al. 2009). Compared to trees subject to defoliation in spring, trees subject to late season defoliation have lower carbohydrates stores (due to light availability and lower temperature) which are required to compensate for winter damage and a shortage of photosynthesis organs resulting in insufficient metabolism to support growth in the new growing season (Floyd and Farrell 2007).

### 3.4.3. Growth recovery ability of *E. bosistoana*

The finding that moderate spring defoliation did not significantly reduce stem or height growth relative to control trees agrees with previous studies which have indicated that up-regulation of photosynthesis (with unchanging ecosystem respiration and increased solar radiation transmissivity) of remaining foliage after a defoliation event can compensate for growth loss (Pinkard et al. 2011a, Elek and Wardlaw 2013). It also reveals that the ability of *E. bosistoana* to recover from defoliaiton in dryland area is comparable to other widely planted *Eucalyptus* species, such as *E. globulus* (Eyles et al. 2009) and *E. nitens* (Elek 1997). This suggests that if moderate spring defoliation is predicted, control is probably not necessary because it is not likely to cause production loss in the long term. However, since defoliated tree can possibly attract more insect attack (Kendrick and Raffa 2006) and repeated defoliation in a season will reduce tree growth, on-going monitoring should be employed to prevent further defoliation. In dryland areas where trees grow slower due to water deficits (Osorio et al. 1998), the impact of defoliation, especially late summer defoliation, may not have been fully revealed. Further measurements on the trees defoliated in this study will be taken to determine if growth does eventually catch up with the growth of control trees, but the time required is beyond that of any thesis as tree have a long rotation period. Nevertheless, these results suggest that control practices (e.g. chemical or biological control) should be considered if severe defoliation of more than one moderate defoliation event is predicted, to avoid production loss.

### 3.4.4. Appropriateness of using artificial defoliation

Using artificial defoliation to predict the impacts of real defoliation by insects is limited because it cannot fully replicate the complexity of plant-insect interactions and chemical exchanges (Baldwin 1990, Zvereva and Kozlov 2014). Several studies have compared the growth and physiological effect of woody plants caused by artificial and real insect defoliation. Results vary but have generally shown that simulation can reflect the direction of tree responses, but can underestimate the effects of real insect defoliation (Britton 1988, Sanchez-Martinez and Wagner 1994, Chen et al. 2002, Quentin et al. 2010). However, this comparison has only been conducted in glasshouse conditions on one *Eucalyptus* species over a short duration. Candy (2000b) claimed that although artificial defoliation may not be accurate enough for physiological studies, it may be enough for decision making in IPM. There are advantages in using artificial defoliation, including the ability to control the severity, pattern, frequency and timing of defoliation to maintain consistency across replicates, as well as avoiding any cage effect resulting from host trees having to be covered in some way in studies managing real insects as defoliation agents (Baldwin 1990). A disadvantage of natural defoliation is that insects may tend to choose trees that have specific properties (for example weaker or more vigorous trees depending on insect species), which may bias the results. Considering these advantages and disadvantages, artificial defoliation was deemed appropriate for this study, but given that it is possible to underestimate the real insect defoliation impact, control action should be considered if severe (about 90%) defoliation is predicted. Careful decisions must be taken if moderate defoliation (about 50%) is predicted and site conditions (e.g. drought) are expected to further suppress tree growth.

## 3.5. Conclusion

Neither moderate nor severe defoliation treatments caused mortality of *E. bosistoana* over experiment period, and trees were recovered with new foliage by the final measurement. Defoliation had greater impact on stem diameter growth than on height growth. Severity, timing and number of defoliation events in a season (repeated defoliation) were important factors driving the impact of defoliation on *E. bosistoana* growth. A high tolerance to 50% defoliation has been found in previous study on other *Eucalytpus* species, while other woody species (such as *P. radiata*) may have a lower tolerance (Pinkard et al. 2017). All defoliation treatments significantly reduced the diameter growth, or both diameter and height growth of *E. bosistoana* except that spring moderate defoliation did not significantly affect both tree diameter and height growth. These results indicated that trees subject to moderate spring defoliation could catch up the growth of control trees, and that the recovery ability of *E. bosistoana* from insect defoliation is comparable or better to other widely planted *Eucalyptus* species. This suggests a single outbreak of *P. charybdis* or/and *O. eucalypti* causing moderate (about 50%) defoliation in spring may not require control action. However, although it was not statistically different, estimated growth rate and final growth increase of spring moderate treatment were lower than that of control trees, so decision making will need further information on final volume loss caused by different levels of defoliation, and the acceptable level of volume loss over the whole rotation. Less than 5% reduction in final volume as an acceptable threshold was used in Pinkard et al. (2015), but a different thresholds can be applied to fit the forest owner's needs. Protecting trees from a severe single defoliation event is important for better control in the future because insect-wounded trees tend to attract more

insect attack and suppressed trees are more likely to suffer from pathogen attack than those undamaged ones, thus a pest monitoring programme is critical to IPM, particularly if one outbreak has already occurred. However, since chemical control has a negative impact on natural enemies of pests, which will impact on future pest control, suitable alternatives and studies on the impact of chemical control on natural enemies in New Zealand eucalypt plantation forestry are recommended.

In contrast to a single moderate defoliation event, severe defoliation (here 90%) at any time, or two moderate or severe defoliation events within the season may require control to prevent significant growth losses. However, since the study site is a particularly dry site which the drought condition may aggravate the impact of defoliation, pest control decisions in other sites need to take the site conditions into account. Furthermore, defoliation levels between 50% and 90% should be investigated taking the environmental conditions into account, since it is not known if these levels of defoliation occurring in spring would significantly reduce tree growth or not. Defoliation above 50% just before winter should be avoided by applying some form of pest control. Pest monitoring is essential for such decision making, but a better understanding of the relationship between defoliation level and insect population size is needed before implementing IPM in this system. The IPM programme for chrysomelid beetles developed by Forestry Tasmania (Candy 2000b, Wardlaw et al. 2010) and the science behind it could be used as an example of how such a study could be undertaken. Using artificial defoliation cannot completely simulate the full strength of real insect defoliation, but it may be good enough to determine the economic threshold for defoliation and is ideal for controlling experimental variables, providing consistency between studies and avoiding bias resulting from insect choice.

Besides severity, number and timing of defoliation events and host species, factors including site conditions, pest species and silvicultural practices (such as fertiliser and irrigation) are also likely to affect the impact of defoliation. There is evidence that considerable variation in defoliation impacts on *Eucalyptus* species is exhibited between sites (Pinkard et al. 2015), so regional or even site-level studies on targeted host and pest species are essential for future understanding of defoliation responses. This is particularly important in stressed environments like dryland areas, because defoliation can amplify the impacts of water stress (McDowell et al. 2008, Pinkard et al. 2011b). Consequently, further studies on defoliation impacts and mitigation measures (e.g. silvicultural practices) should be conducted on more of the *Eucalyptus* species being developed for dryland production in New Zealand to fully understand their specific response in the new environment. Long-term studies covering a full rotation are recommended to determine the cost-benefit potential of an IPM programme in these plantations.

# CHAPTER 4 ASSESSING WITHIN-SPECIES VARIATION OF *E. BOSISTOANA* IN INSECT SUSCEPTIBILITY

## 4.1. Introduction

Environmental awareness and current policy in some countries requires imported products to come from sustainably managed plantations. In recent years, traditional forest management practises have been influenced by these emerging requirements to incorporate more sustainable practices, including integrated pest management (IPM). While post-planting pest management practices like pest monitoring to target susceptible life stages of pests (see Chapter 2) and biological control can successfully reduce the need for traditional chemical control, preventative measures such as selecting insect resistant and/or tolerant species or genotypes could be more efficient, and significantly reduce management cost in the long-term.

Although pest resistant genotypes are used in many crop systems, they are relatively rare for plantation forestry. Tree breeding programmes generally target other traits, such as growth rates and wood properties, whereas insect pests are usually managed using chemical, or occasionally, biological control. This is because unlike agricultural crops such as rice and maize, trees are generally not killed by insect pests, and reduced tree growth resulting in lower or slower production of timber, is easier to overlook than complete loss of saleable products like grains. Although trees can tolerate relatively high levels of damage (Henery 2011, Pinkard et al. 2011a; Chapter 3), ignoring the potential impacts of insect pests when developing a new forest resource can result in significant pest management costs later on, or even lead to a failure to establish the new resource. For example, the success of previous attempts to establish large scale *Eucalyptus* plantations in New Zealand was limited, partly because of insect pests (particularly, the *Eucalyptus* tortoise beetle *Paropsis charybdis* to the development of *E. nitens*) and diseases originating from Australia (Clark 1930, Murray et al. 2008, Apiolaza et al. 2011b). Similarly, during the first and second phase of the Three-North Shelterbelt Programme in China, lack of consideration of insect pest issues led to 80% of the poplar stands planted being damaged by poplar longhorn beetles (multiple Cerambycid species, including *Anoplophora glabripennis* (Motschulsky), *Apriona germari* Hope and *Batocera horsfieldi* (Hope) (Coleoptera: Cerambycidae)) (Ji et al. 2011). Subsequently, mixed plantings of resistant tree species with other species were used to mitigate the pest problem successfully (Ji et al. 2011). Pest issues in forestry have become increasingly prevalent due to growing numbers of unexpected new insect incursions on a global scale and unknown insect responses to predicted climate change scenarios (Henery 2011, Wingfield et al. 2013).

Previous studies have shown some positive examples of planting resistant trees in plantations. Breeding sitka spruce (*Picea sitchensis* (Bong.) Carr.) resistant to white pine weevil (*Pissodes strobi* Peck) is one example of a plantation forestry programme that specifically aimed to breed for insect pest resistance, and which proved to be a more effective pest management method than traditional silvicultural controls (King and Alfaro 2009, Hall et al. 2011). Selecting pest resistant clones of *E. grandis* x *E. urophylla* hybrids has increased planting stock and forest health in Brazil and South Africa (Wingfield et al., 2013). Insect resistance was also considered in a commercial breeding programme of willows started in 1987 (Larsson 1998). However,

Henery et al. (2011) summarised the difficulties in selecting insect resistant eucalypts. They noted that insect resistance was overlooked compared to tree growth traits, there may be a trade-off between traits for fast growth and defensive traits. Also, they pointed out that selection for pest resistance could lead to decrease in genetic diversity, which could increase the risk of future insect outbreaks of both existing and the increasing number of newly introduced insect pests. There was also risk of insect pests switching from preferred hosts to species they previously ignored, because they may develop an ability to feed on such species over time.

New technologies such as DNA-based tools for identification, detection and monitoring of pests, and progress in methods for breeding and selecting less susceptible species and hybrids have given *Eucalyptus* plantations an optimistic future (Wingfield et al., 2013). Recent studies have demonstrated that instead of breeding specifically for resistant genotypes, screening existing breeding trials for heritable variation in insect *tolerance* (ability of trees to recover growth from insect defoliation) and eliminating the most pest susceptible genotypes from those with other desirable traits for maximum growth or wood properties, can be a feasible and more cost effective method of tree improvement (Henery 2011, Elek and Wardlaw 2013, Boshier and Buggs 2015). Tree improvement by eliminating insect-susceptible genotypes or selecting tolerant genotypes could significantly reduce economic losses from insect pests as part of an environmentally sustainable IPM strategy over the long term (Elek and Wardlaw 2013).

*Eucalyptus bosistoana* has been recorded to suffer severe defoliation by *P. charybdis* in New Zealand. It is within the subgenus *Symphyomyrtus*, which is generally considered to attract more insect attack than the subgenus *Eucalyptus* (previously subgenus *Monocalyptus*) (e.g. *E. globoidea*). *Eucalyptus nitens* and *E. globulus* are also *Symphyomyrtus* and the former is very attractive to insect attack (Noble 1989). However, *Eucalyptus* presents great variation in crown and foliar morphology, leaf chemistry and growing strategy, which in combination influence the susceptibility to insects both within and between species. For example, concentrations of plant secondary metabolites, which are important in constitutive defence, vary between and within eucalypt species (Eyles et al., 2013). *Eucalyptus bosistoana* families in the NZDFI trials exhibit different crown and foliar morphology, and various levels of insect damage have been observed on individual trees, indicating potentially heritable differences in insect susceptibility between families. Consequently, screening the existing *E. bosistoana* in the established NZDFI trials for the most and least pest-susceptible families, will assist the programme in selecting suitable future stock for breeding, and improve the plantation productivity by reducing the risk of insect outbreaks and meanwhile minimising the use of chemical control. This will also benefit the industry by providing the foundation for a strong IPM programme over the long-term.

## 4.2. Objectives

The main objective of this chapter is to determine if there is significant variation in the insect susceptibility of 14 *E. bosistoana* families and 1 *E. globoidea* family, and which of these families are the most/least susceptible to the key insect defoliators in the dryland eucalypt plantation. The second objective is to identify the most effective and efficient assessment method for examining insect susceptibility of different eucalypt genotypes. To resolves these objectives, a tree health assessment trial was conducted. Four health assessment methods were

used to determine indices of insect susceptibility and then compared to find out if the methods were equivalent in their predictions and more or less practical in the field. The breeding programme will benefit from screening insect susceptibility of different families, such that more tolerant families (those for which tree growth is less significantly affected by insect defoliation even if damage occurs) of those already selected for fast growth and elite wood properties can be maintained in the programme, while the most susceptible families can be removed. In the long term, planting less susceptible stock will reduce insecticide use and production loss caused by pest damage. Results from comparing different assessment methods will show which methods are the most effective and efficient for assessing insect damage in future research and forest management.

## 4.3. Methodology

### 4.3.1. Study site and assessed families

To assess variation in insect susceptibility within *E. bosistoana* families (family = seeds collected from the same known mother tree), an extensive health assessment was conducted at the same site as in Chapter 2, comprising 1750 trees. The breeding trial was established to compare growth performance of 40 *E. bosistoana* families from Victorian provenances, Australia. Since it was not feasible to assess insect susceptibility of all 40 families, 12-17 replicates of 14 families (from three different provenances) were selected (Figure 4.1). Initially, 221 trees were selected to represent the 14 *E. bosistoana* families and one family of *E. globoidea,* which was included for comparison as it is the only family within the subgenus *Monocalpytus* represented in this durable eucalypt trial. *Monocalyptus* are often considered to be less susceptible to insect pests (Li 1994, Stone et al. 1998). Fourteen more trees were added early in the trial to increase replication and capture within-family variation in tree height. Three trees were eliminated from the study as they became unhealthy (probably due to drought) (Table 4.1).

| Family | Provenance |
|---|---|
| 104 | Marulan |
| 108 | Marulan |
| 111 | Marulan |
| 114 | Marulan |
| 116 | Marulan |
| 121 | Marulan |
| 125 | Bungonia |
| 128 | Bungonia |
| 129 | Bungonia |
| 130 | Bungonia |
| 133 | Southern |
| 134 | Southern |
| 135 | Southern |
| 138 | Southern |

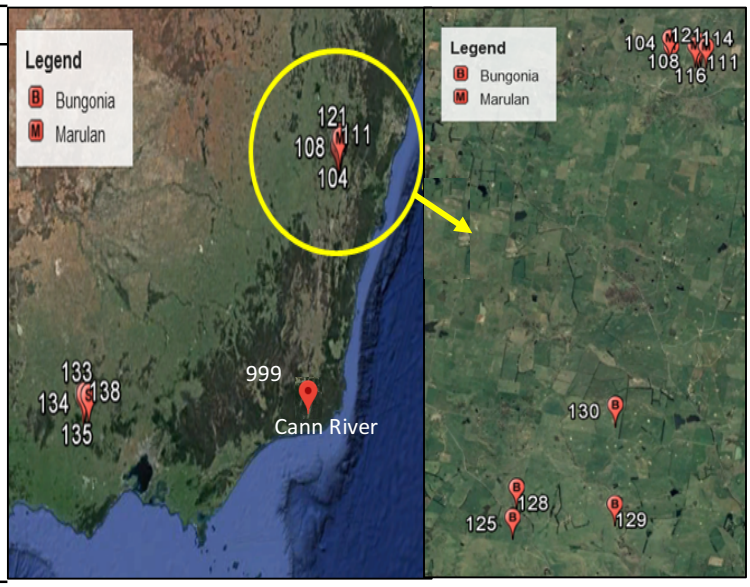



**Figure 4.1** Provenance locations within Australia for the 14 *E. bosistoana* families assessed for insect susceptibility in Marlborough, New Zealand. Bungonia is approximately 10km south of Marulan and 800km north of the 'Southern' provenance.

### 4.3.2. Defoliation damage assessment

Four methods were used to estimate susceptibility of *E. bosistoana* families to four key insect pests (*Paropsis charybdis*, *Opodiphthera eucalypti*, *Strepsicrates macropetana* and *Phylacteophaga froggatti*). Two methods were based on shoot assessment and two methods on whole tree level assessment:

1. Pest counting per shoot

2. CDI (tree)

3. CDI (shoot)

4. Tree grading

For the pest counting method, the number of different life stages of each of the four pest species were counted on 3-5 selected shoots of assessed trees. The detailed method has been described in Chapter 2 (section 2.2.1.4).

The second method, CDI (tree), applied the standard Crown Damage Index (CDI) method (Stone et al. 2003). The defoliation caused by the above insects was assessed by visually estimating the proportion of defoliation of the tree crown (incidence) and the proportion of damage per damaged leaf (severity). This is the most common method for assessing defoliation of eucalypts in Australia. Each selected tree was given three CDI scores representing natural damage caused by the different guilds of insect herbivores as follows: 1) chewing damage (caused by *P. charybdis* and *O. eucalpyti*), 2) leaf roll damage (*caused by S. macropetana*) and 3) mining damage (caused by *Ph. froggatti*). The CDI score is determined as;

$$\text{Health score} = (\text{Incidence x Severity}) \times 100\%$$

Where *incidence* is the estimated proportion of damaged leaves per tree and *severity* (Figure 4.2) is the average proportion of damage to each leaf.

The CDI (shoot) method was a modified version of the CDI (tree) method, in which the examined units were shoots instead of trees. The CDI (shoot) score for each tree was calculated as the average CDI of the 3-5 shoots assessed per tree.

The tree grading method was only used for assessing the chewing damage (caused by *P. charybdis* and *O. eucalypti*). Damage assessment was achieved by visually inspecting the whole tree crown. Each tree was given a damage grade, which was recorded as a) little or no damage, b) light damage, c) moderate damage and d) moderately severe damage.

The pest counting assessment was conducted on all 12 sampling occasions (which occurred at intervals of ~3-4 weeks from November 2015 to April 2017 except between April and September 2016) as it was also used to monitor population dynamics of the four defoliators in Chapter 2. The other three methods were conducted between 2 and 4 times over the experiment period (Table 4.1). Susceptibility to *P. charybdis* was assessed on all sampling occasions using all four methods. Susceptibility to *O. eucalypti* was only assessed using the pest counting method, because the damage produced by the species is visually similar to that of *P. charybdis*, and of the two species *O. eucalypti* was observed to contribute little to overall levels of chewing damage. The CDI (tree) assessment was only conducted for *S. macropetana* and *Ph. froggatti* in December 2015. CDI (shoot) assessments for *S. macropetana* were not analysed because the old damage of *S. macropetana* was difficult to reliably distinguish from wind and/or possible

abiotic damage such as pathogens. In contrast, CDI (tree) assessment for *S. macropetana* in December 2015 was analysed because only obvious and recent damage recorded.

Tree height and stem diameter at 10 cm from the ground were measured on three occasions, February 2016, January 2017 and April 2017. However, since markers showing stem measurement points faded, repeated measurements of stem diameter were not considered accurate enough for analysis, and only tree height will be presented in the result section. Relative proportion of flush, expanding leaves and mature leaves per shoot were used in statistical analysis, and detail method was in Chapter 2 (section 2.2.1.3).

**Table 4.1** Number of trees assessed from each family on each sampling occasion. Superscript beside each month shows the assessment methods used during that assessment period: 1) pest counting method; 2) CDI (tree) method; 3) CDI (shoot) method; 4) tree grading method.

| Family | 2015 | | 2016 | | | | | | 2017 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Nov[1,3] | Dec[1,2,3] | Jan[1] | Feb[1,3] | Mar[1,3] | Early Oct[1] | Late Oct[1] | Dec[1] | Jan[1,2,4] | Feb[1] | Mar[1] | Apr[1,2,4] |
| 104 | 15, | 15, | 15, | 15, | 15, | 15, | 15, | 15, | 15, | 15, | 15, | 15 |
| 108 | 14, | 15, | 15, | 15, | 15, | 16, | 16, | 16, | 16, | 16, | 16, | 16 |
| 111 | 15, | 15, | 15, | 15, | 15, | 15, | 15, | 15, | 15, | 15, | 15, | 15 |
| 114 | 16, | 16, | 16, | 16, | 16, | 16, | 16, | 16, | 16, | 16, | 16, | 16 |
| 116 | 15, | 15, | 15, | 15, | 15, | 15, | 15, | 15, | 15, | 15, | 15, | 15 |
| 121 | 12, | 15, | 15, | 15, | 15, | 15, | 15, | 15, | 15, | 15, | 15, | 15 |
| 125 | 16, | 16, | 16, | 17, | 17, | 17, | 17, | 17, | 17, | 17, | 17, | 17 |
| 128 | 13, | 15, | 15, | 15, | 15, | 16, | 16, | 16, | 16, | 16, | 16, | 16 |
| 129 | 15, | 15, | 15, | 15, | 15, | 15, | 15, | 15, | 15, | 15, | 15, | 15 |
| 130 | 15, | 15, | 15, | 16, | 16, | 15, | 15, | 15, | 15, | 15, | 15, | 15 |
| 133 | 17, | 17, | 17, | 17, | 17, | 17, | 17, | 17, | 17, | 17, | 17, | 17 |
| 134 | 15, | 15, | 15, | 16, | 16, | 15, | 15, | 15, | 15, | 15, | 15, | 15 |
| 135 | 15, | 15, | 15, | 15, | 15, | 15, | 15, | 15, | 15, | 15, | 15, | 15 |
| 138 | 15, | 15, | 15, | 15, | 15, | 15, | 15, | 15, | 15, | 15, | 15, | 15 |
| 999 | 13, | 15, | 15, | 16, | 16, | 15, | 15, | 15, | 15, | 15, | 15, | 15 |
| Total | 221, | 229, | 229, | 233, | 233, | 232, | 232, | 232, | 232, | 232, | 232, | 232 |

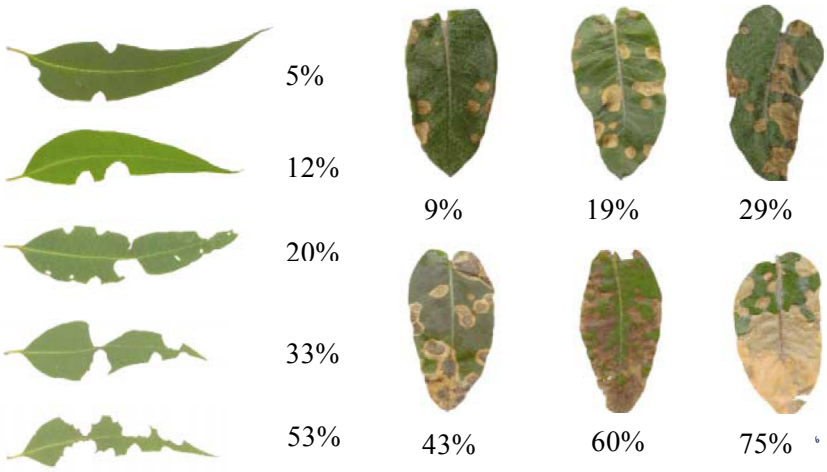



**Figure 4.2** Examples of variation in the estimated severity of chewing damage (left) and mining damage (right). Figure reproduced from Stone et al. (2003).

### 4.3.3. Statistical analysis

All data analysis were conducted in R (R Development Core Team 2008). Package lme4 (Bates et al. 2015) was used to build generalised linear mixed-effects models (GLMMs) with Poisson as family, to assess the effect of tree family, tree height, time (repeated measurements) and relative proportion of flush, expanding leaves and mature leaves on larval abundance of *P. charybdis*, larval and egg abundance of *O. eucalypti*, larval abundance of *S. macropetana*, and abundance of *Ph. froggatti* mines. Impact of other life stages of these insect species were not assessed, either because counts were too low for statistical rigour, or because the life stage was not considered key to identifying host preference (e.g. non-feeding stages). Function lmer was used to construct linear mixed effect models (LMMs) to assess the effect of family, tree height, time (repeated measurements) and relative proportion of flush, expanding leaves and mature leaves on the CDI (shoot) scores. LMMs were also constructed to test the effects of family, tree height and time on the CDI (tree) scores. Residuals plots were checked for homogeneity. In contrast, GLMMs with binomial family were used to test for effects of *Ph. froggatti* as measured using the CDI (shoot) method because the large number of zero values in the data made the above models inappropriate. Percentage CDI values were transformed using a power-scale function FindLambda (provided by E. G. Mason, School of Forestry, University of Canterbury), which was used to search for the value of lambda which made the frequency distribution as normal as possible. For the tree grading method, cumulative link mixed models (CLMMs) were built with function clmm to analyse the effect of tree family, tree height and time (repeated measurements) on insect damage, analysed using the ordinal R package (Christensen 2015).

Model selection for these analysis followed the process in Zuur et al. (2009) and Bolker et al. (2009): 1) the beyond optimal model (models with the fixed effects part containing all explanatory variables and as many interactions as possible) was used to find the optimal structure of the random effects part. Each factor was dropped in turn and the optimal model was selected using the likelihood ratio statistic and Akaike information criterion (AIC). R function anova and AIC were used for model selection; 2) After the optimal random structure was found, the optimal fixed structure was found by dropping each explanatory variable in turn and the anova and AIC functions used to select the best-fitting model. The fixed effects part of the model quantified the overall effects (across all families) of tree height, time and proportion of different leaf age types. The random effects part quantified the variation across families and

populations of the fixed-effect parameters. Factors remaining in the final models (Table 4.2) were the factors that had significant effects on the response variables (pest abundance, defoliation percentage and defoliation grading respectively for pest counting, CDI and tree grading methods). For pest counting, CDI (tree) and CDI (shoot) methods for *P. charybdis*, and pest counting method for *S. macropetana*, the models with time × family in the random effects part were the best-fitting models, but results of models with family (without interaction with time) in the random effects part will also be presented to show the overall average effect of family on pest load and defoliation percentage.

For each assessment method, families were ranked from the most to least insect-susceptible based on the pest load or defoliation level observed. Rankings were compared by testing for correlations between each pair of methods using cor function in base R.

**Table 4.2** Statistical models using in analysing the results from different health assessment methods for each insect defoliator, and factors in the fixed and random parts in the final (best-fitting) models from model selections. Symbol '×' indicates an interaction effect on the response variable; Symbol '+' indicates no interaction effect; plot/tree/shoot stands for effect of shoot nested within tree nested within plot. ([1]random part of the final best-fitting model of the analysis on specific health assessment method; [2]random part of the model showing the overall family effect.)

| Insect | Assessment method | Statistical model | Fixed part | Random part |
|---|---|---|---|---|
| *P. charybdis* | Pest counting | GLMM with Poisson family | tree height × time + flush × expanding leaves | [1]time × family, plot/tree/shoot |
| | | | tree height × time + flush × expanding leaves | [2]family, plot/tree/shoot |
| | CDI (tree) | LMM | tree height + time | [1]time × family, plot/tree |
| | | | tree height + time | [2]family, plot/tree |
| | CDI (shoot) | LMM | tree height + time + expanding leaves | [1]time × family, plot/tree/shoot |
| | | | tree height + time + expanding leaves | [2]family, plot/tree/shoot |
| | Tree grading | CLMM | tree height + time + family | plot/tree |
| *O. eucalpyti* | Pest counting | GLMM with Poisson family | tree height × time + flush + expanding leaves | plot/tree/shoot |
| *S. macropetana* | Pest counting | GLMM with Poisson family | tree height × time + flush + expanding leaves | [1]time × family, plot/tree/shoot |
| | | | tree height × time + flush + expanding leaves | [2]family, plot/tree/shoot |
| | CDI (tree) | LMM | tree height | plot/tree |
| *Ph. froggatti* | Pest counting | GLMM with Poisson family | tree height + time | plot/tree/shoot |
| | CDI (tree) | LMM | mature leaves | family, plot |
| | CDI (shoot) | GLMM with binomial family | tree height, time × mature leaves | family, plot/tree/shoot |

## 4.4. Results

### 4.4.1. Tree height and emergence of new leaves

At the first measurement, tree height ranged from 1.15 m to 5 m (average 2.7 m). Tree height of families from the Marulan and Bungonia provenances were not significantly different from each other, with the exception of family 125, which was significantly taller than other families from both provenances. *Eucalyptus bosistoana* families from the Southern provenance and the single *E. globoidea* family, were significantly taller than *E. bosistoana* families from the other two provenances, but not significantly different from each other (Figure 4.3).

Foliage flush of Southern provenance families was delayed relative to other families in season 1, with flush peaking in December, compared to November for Bungonia and Marulan provenances (Figure 4.4). Phenology was more synchronised in season 2, with the highest proportions of flush occurring in early December for all *E. bosistoana* provenances (Figure 4.4a). The proportion of expanding leaves peaked in November for the Marulan and Bungonia families and in January for the Southern families (Figure 4.4b). In season 2, the highest proportion of expanding leaves of three provenances appeared together in late December. In season 2, families from the Southern provenance had a lower proportion of both flush and expanding leaves than the families from Bungonia and Marulan.

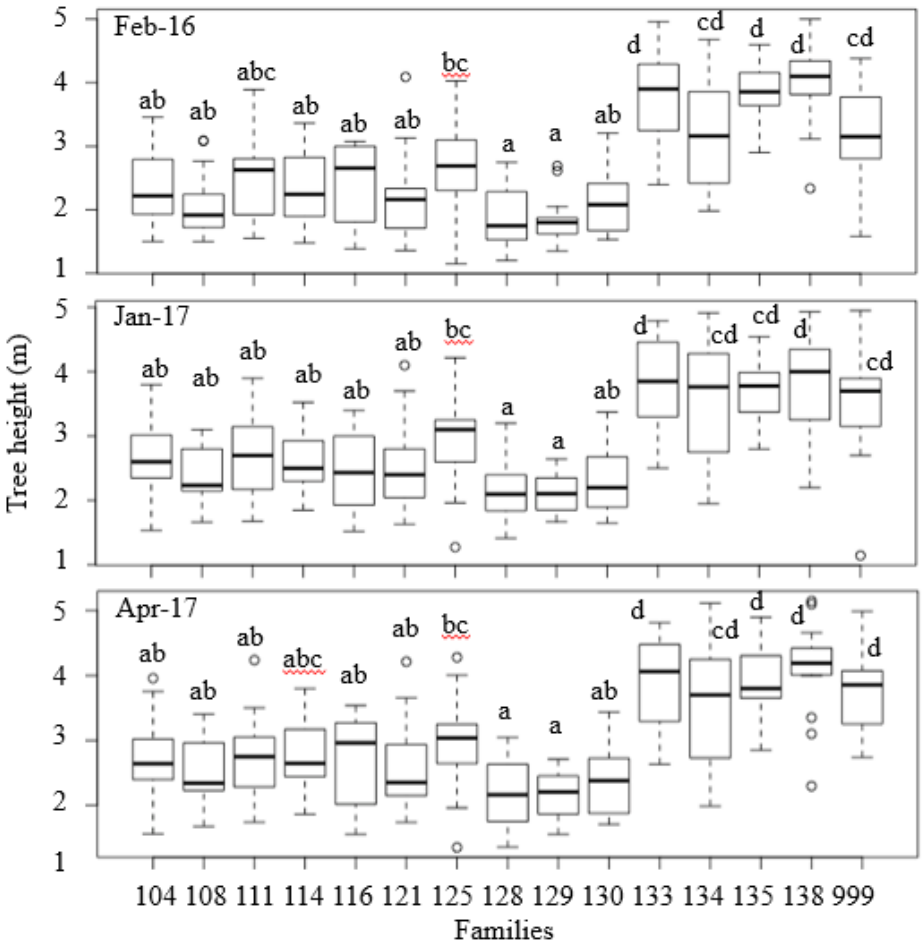



**Figure 4.3** Average tree height of 14 *E. bosistoana* families and 1 *E. globoidea* (999) family measured in February 2016, January and April 2017. Lower case letters show the pairwise comparison of least squares means of families (families sharing a letter are not significantly different).

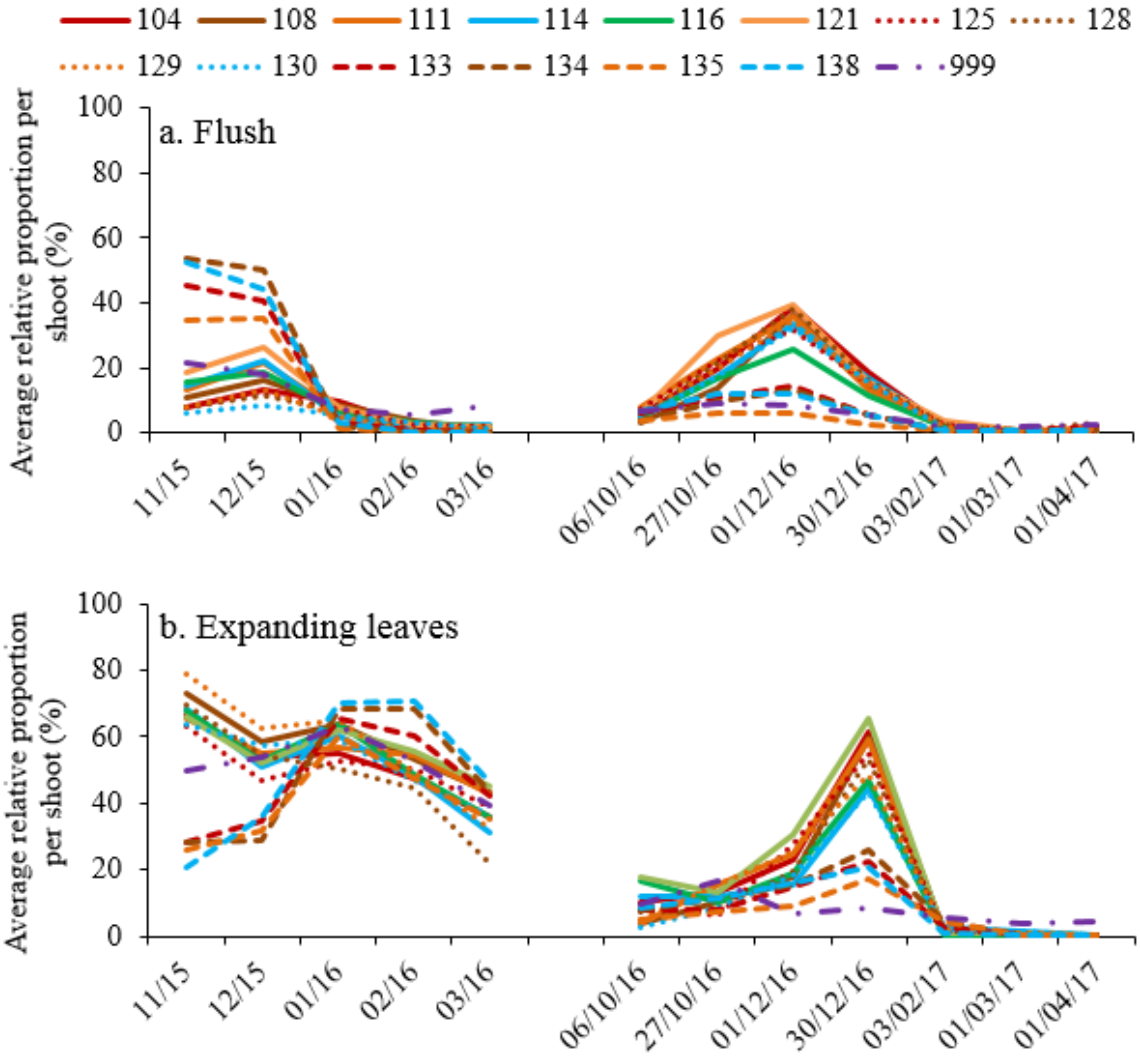



**Figure 4.4** Monthly variation in the average relative proportion of a) flush, and b) expanding leaves per shoot of 14 *E. bosistoana* and 1 *E. globoidea* (999) families over two growing seasons.

### 4.4.2. Susceptibility of *E. bosistoana* families to *P. charybdis*

#### *4.4.2.1. Pest counting method*

The families with the highest *P. charybdis* abundance varied depending on life stage (egg batches, larvae and adults). Overall, family 133 and 138 had the highest average abundance of adults and larvae. Since the adult stage of *P. charybdis* is highly mobile, their presence on a tree is not necessarily indicative of feeding or oviposition preference. As such, the presence of egg and larval stages are better indicators of host preference. Egg batches of *P. charybdis* were rarely observed over the experiment period. The highest abundance was observed in November 2015 on families 121 and 111. No egg batches were found on families 108, 125, 128, 134, 138 and 999 during the experiment period. Larvae were only sufficiently abundant to assess host preference on five sampling occasions: November and December 2015, early and late October and early December 2016 (Figure 4.5). Since abundance of egg batches was too low for

analysis, ranking the observed pest load of families to *P. charybdis* was based on larval abundance only.

Over the two complete sampling seasons, larvae were most abundant on Southern provenance families 133, 138, 134 and 135 (Figure 4.5), while families 108 (from provenance Marulan) and *E. globoidea* had the lowest abundance. However, several families from other provenances (e.g. 104, 111, 121 and 130) had pest loads as high as or higher than the Southern provenance in at least one month. Larval abundance on other families was inconsistent across months. Peak abundance occurred in November 2015 and late October 2016 and was greatest on, families 138, 134, 133 and 121. Larvae abundance on *E. globoidea* (family 999) remained low through both seasons, but several *E. bosistoana* families had similar or lower larvae abundance in all months.

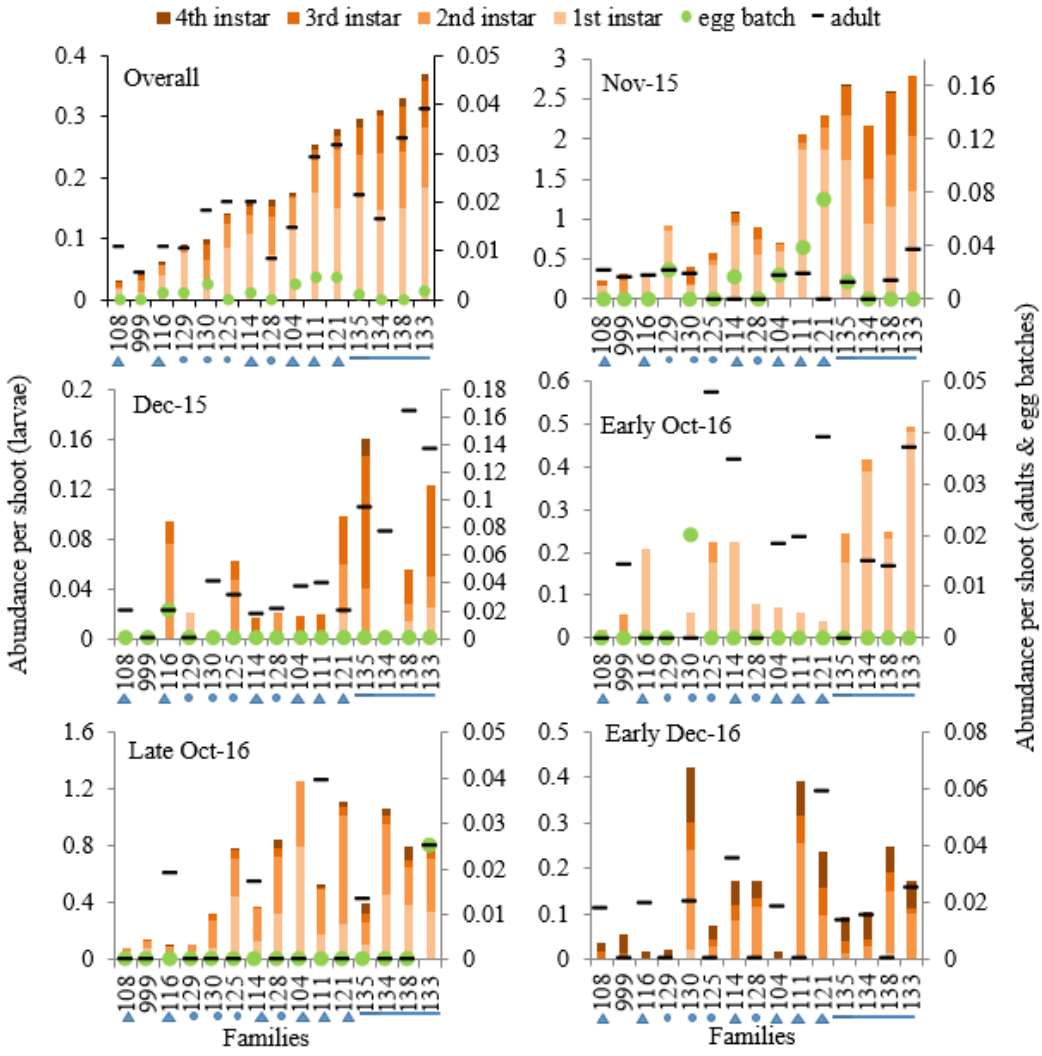



**Figure 4.5** Abundance of egg, larval and adult stages of *P. charybdis* per shoot for each of 14 *E. bosistoana* and 1 *E. globoidea* families during the 5 peaks and overall average for these months. Families are ordered based on total average larval abundance over the entire sampling period. Left y-axis = abundance of larvae per shoot, right y-axis = abundance of egg batches and adults per shoot. Provenances: – Southern, ● Bungonia, ▲ Marulan.

The fixed part of the final GLMM model (Table 4.2) of pest counting method on *P. charybdis* larvae abundance indicated that abundance increased with tree height and increasing proportions of flush and expanding leaves. These effects were significant for all months except the tree height effect in Dec-15. The random part of the model reflected variations larval abundance among families which were not explaned by factors in the fixed part of the model. The random part indicated that family had a significant effect on larval abundance (Figure 4.6), and time had a significant effect on the effect of family. As such family ranking (pest load relative to all other families) for pest abundance varied between months (Figure 4.6b-f). Over all survey months combined families 111, 121 (Marulan provenance) and 133 (Southern provenance) were ranked the highest (had a higher pest load relative to other families) among families (Figure 4.6). Families with relatively low larval abundance included 116 (Marulan provenance) and 999 (*Monocalpytus*). Results from both models showed that pest load of families from Bungonia and Marulan provenance could be above or below average, but the families from the Southern provenance were consistently above average (except 135, late Oct-16).

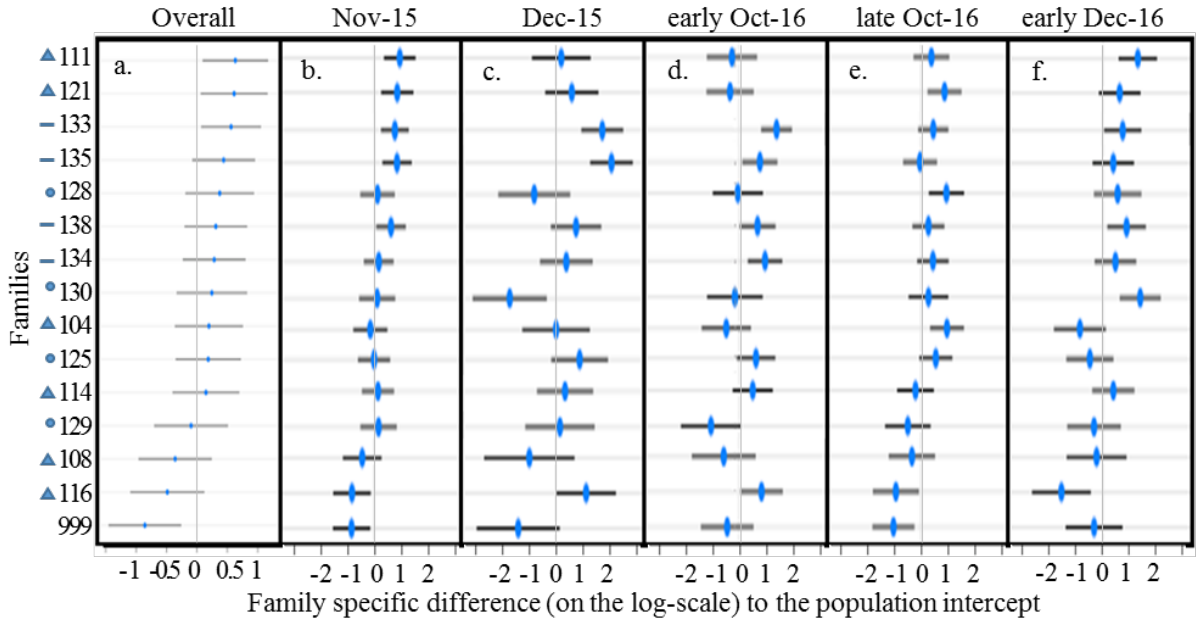



**Figure 4.6** Random effects of family. Difference (± 95% Cl) of family-specific parameter values from the overall average larval abundance (population intercept, shown as 0) predicted by the GLMMs constructed for the pest counting method: a) over all assessed occasions (model with family random effect without interaction with time); b - f) on different assessment occasions (model with family random effect as family × time). Families are ordered based on the predicted family specific difference to the population average (from top to bottom was the family with the most larval abundance to the family with the least larval abundance). Positive values = greater pest load than the overall average vs. negative values = less pest load than the overall average. Family provenance = – Southern, ● Bungonia, ▲ Marulan.

### *4.4.2.2. CDI (tree) method*

Defoliation assessment for *P. charybdis* using the CDI (tree) was conducted in December 2015 (season 1), January and April 2017 (season 2). Defoliation was greatest in April. Percentage of

chewing damage to the tree crown was under 50% for all trees (Figure 4.7). Variations in the relative rank of families were smaller than observed using the pest counting method. Families with the most damage (133, 134, 135 and 138) were from the Southern provenance, sustaining around 10% to 30% defoliation, followed by family 128. The rankings of families from the other provenances varied over the season but all generally sustained damage of <5%. The single *Monocalyptus* family (999) performed well, with less than 5% damage, but 9 *E. bosistoana* families performed better than it.

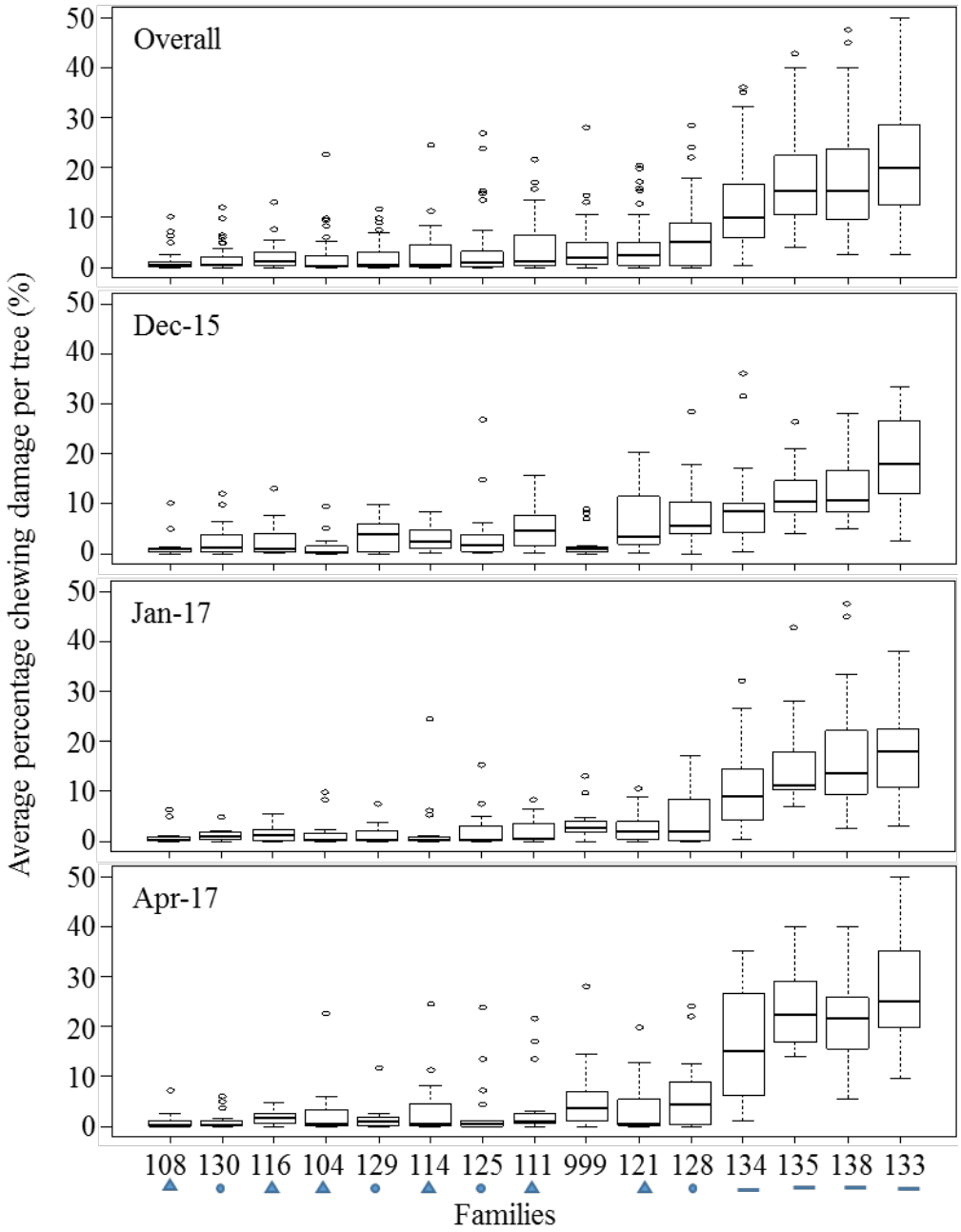



**Figure 4.7** Average percentage of tree crowns of each eucalypt family damaged by *P. charybdis* chewing in December 2015, January and April 2017. Families are ordered of based on the overall average defoliation across the sampling entire period. Family provenance = – Southern, ● Bungonia, ▲ Marulan.

The fixed part of the final LMM model for the CDI (tree) method assessment of *P. charybdis damage* (Table 4.2) indicated that tree height and time significantly affected the percentage of chewing damage. Chewing damage percentage and tree height were positively correlated. Greatest damage was present in December 2015, which was significantly greater than January 2017 but not significantly different from the April 2017 assessment. The random part of the final model on CDI (tree) indicated that family had significant effects on chewing damage percentage, and the effect varied between months (Table 4.2, Figure 4.8 b-d). Result from the model with family effect (without interaction with time) which indicated the overall effect of family, showed that the Southern provenance families (133, 138, 135 and 134) and 128 (Bungonia provenance) sustained the highest level of damage (Figure 4.8a), and this was true in all months. Performance of families from other provenances varied by month but they generally suffered less than average damage. The *Monocalyptus* family (999) performed better than seven *E. bosistoana* families, and had less than average damage in December 2015, but higher than average damage in January and April 2017. Family specific differences to the overall average CDI were highly similar in the two season 2 assessments (Figure 4.8c & d).

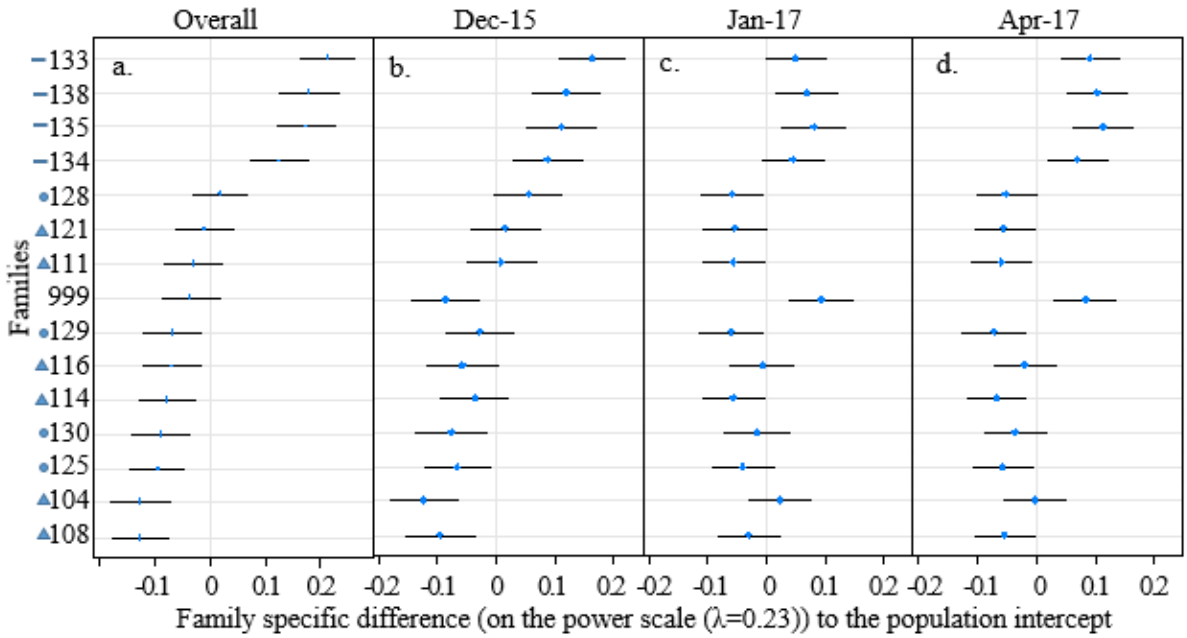



**Figure 4.8** Random effects of family. Difference (± 95% Cl) of family-specific parameter values from the overall average chewing damage per tree (shown as 0) predicted by the LMMs constructed for CDI (tree) method: a) over all assessed occasions (model with family random effect without interaction with time); b - f) on each separate assessment occasion (model with family random effect as family × time). Families are ordered based on the family specific difference to the population average (with the most defoliated family at the top to the least defoliated family at the bottom). Positive values = greater defoliation than the overall average vs. negative values = lower defoliation than the overall average. Family provenance = – Southern, ● Bungonia, ▲ Marulan.

### *4.4.2.3. CDI (shoot) method*

Percentage chewing damage per shoot was generally found to be under 50%, but occasionally over 80% (**Error! Reference source not found.**). Using the CDI (shoot) method, family ranks

emained relatively consistent across the season. Greatest chewing damage was always observed on the Southern provenance families (133, 134, 135 and 138) and family 121 (Bungonia provenance). Family 133 sustained the most damage in three of the four assessments. Family 121 and 111 were ranked as the 5th and 6th most damaged families for all months. Other families sustained minimal damage and also generally had less variation in average damage across the seasons. Several *E. bosistoana* families sustained less chewing damage on average than the monocalypt *E. globiodea* family 999 (**Error! Reference source not found.**, ov and Dec-15).

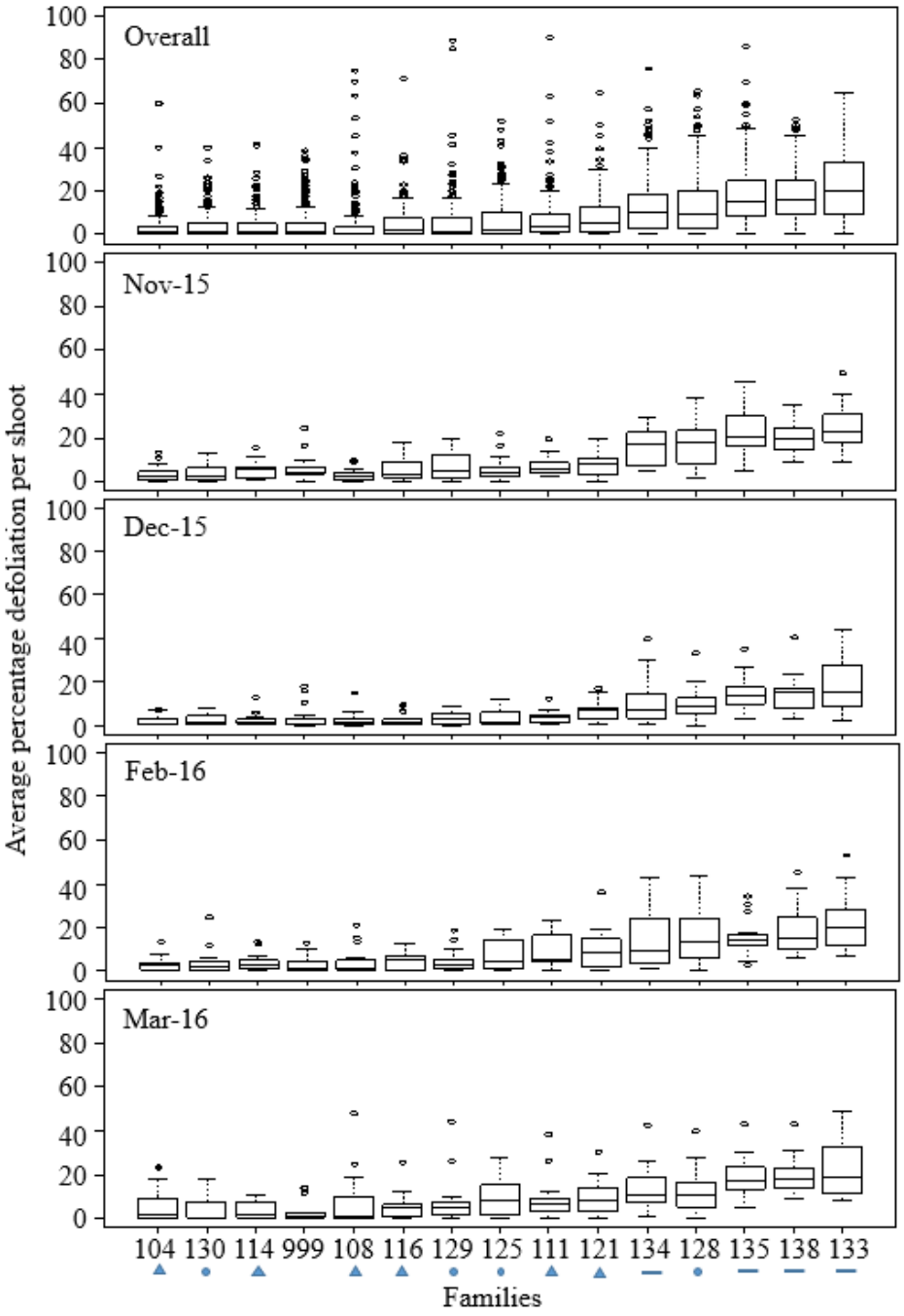



**Figure 4.9** Average percent defoliation per shoot for each family in November and December 2015, and February and March 2016. Order of families is based on overall percentage defoliation per shoot. Family provenance = – Southern, ● Bungonia, ▲ Marulan.

The fixed part of the final model (Table 4.2) for CDI (shoot) indicated that tree height, time and the amount of expanding leaves significantly affected the percentage of shoot damage. Chewing damage and tree height were positively correlated. Greatest damage was observed in November 2015, and was significantly greater than other months. The random effect part of the final model indicated the family effect on shoot damage varied between months (Figure 4.10b-e), but the most defoliated families were consistent. These were the Southern provenance families and 128 (Figure 4.10), similar to the results from CDI (tree) method (Figure 4.8). These families always suffered from more than average damage in all months. Other families

performed similarly, either close to or less than average percentage shoot damage. Overall, the *Monocalyptus* family (999) sustained the least shoot damage.

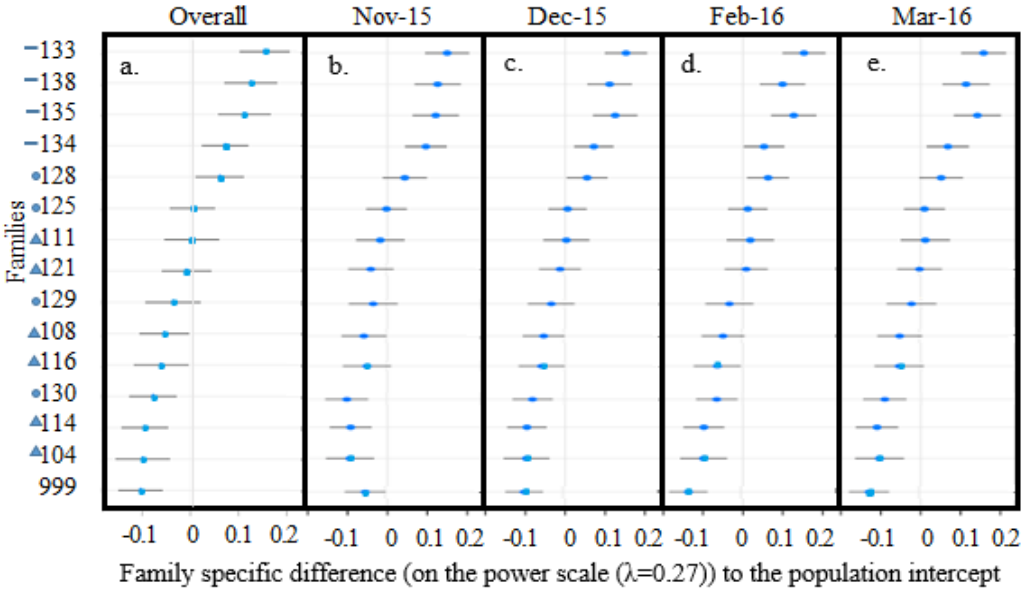



**Figure 4.10** Random effects of family. Difference (± 95% Cl) of family-specific parameter values from the overall average percent chewing damage per shoot (shown as 0) predicted by the final LMMs constructed for the CDI (shoot) method: a) over all assessed occasions (model with family random effect without interaction with time); b – e) on each separate assessment occasion (model with family random effect as family × time). Families are ordered based on the family specific difference to the population average (with the most defoliated family at the top to the least defoliated family at the bottom). Positive values = greater defoliation than the overall average vs. negative values = lower defoliation than the overall average. Family provenance = – Southern, ● Bungonia, ▲ Marulan.

### *4.4.2.4. Tree grading method*

Family rankings for pest damage using the tree grading method were relatively consistent between months (Figure 4.11). Families 133, 134, 135 and 138 (all Southern provenance) had the highest level of chewing damage, followed by 128, 999 then 121. Families with the least damage were 108, 130 and 129. More than half of the trees within each Southern provenance family were classified as having moderate or moderately-severe damage. Families 125, 121, 128 and 999 included individual trees varying from no damage to moderately severe damage, while other families consistently sustained either more light (level a & b) or more severe (level c & d) damage.

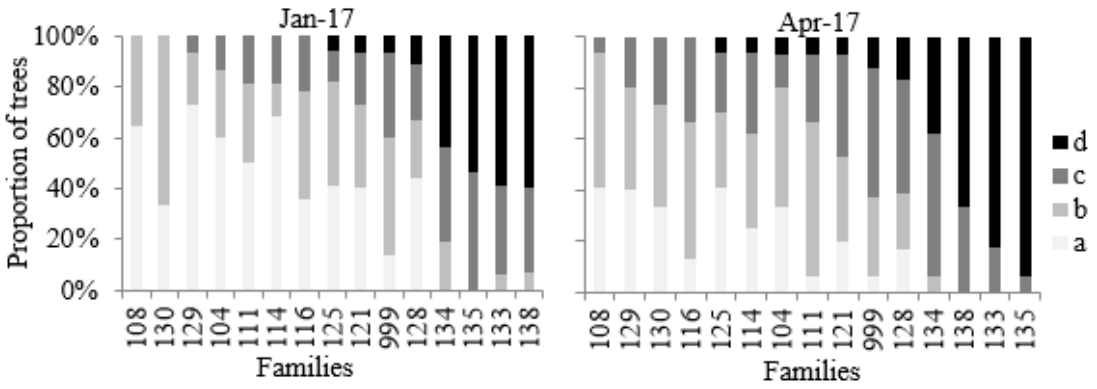



**Figure 4.11** Proportion of individual trees per family assigned to each level of chewing damage (a = no or little chewing (<5%); b = light chewing (5–25% defoliation); c = moderate chewing (26-40% defoliation); d = moderately severe chewing (> 40% defoliation).

Analysis of CLMM model (Table 4.2) showed that family, tree height and assessment time significantly affected damage level. Trees assessed in April 2017 sustained greater damage than in January 2017 (Figure 4.12a). Damage level and tree height also had a positive significant correlation (Figure 4.12b). The Southern provenance families again sustained the greatest damage, with 80% - 90% of trees suffering moderate to moderately severe defoliation. Over 50% of trees sustained moderate to moderately severe defoliation in family 128 and 999. In contrast, > 50% of trees in families 108, 129 and 104 suffered little or no defoliation.

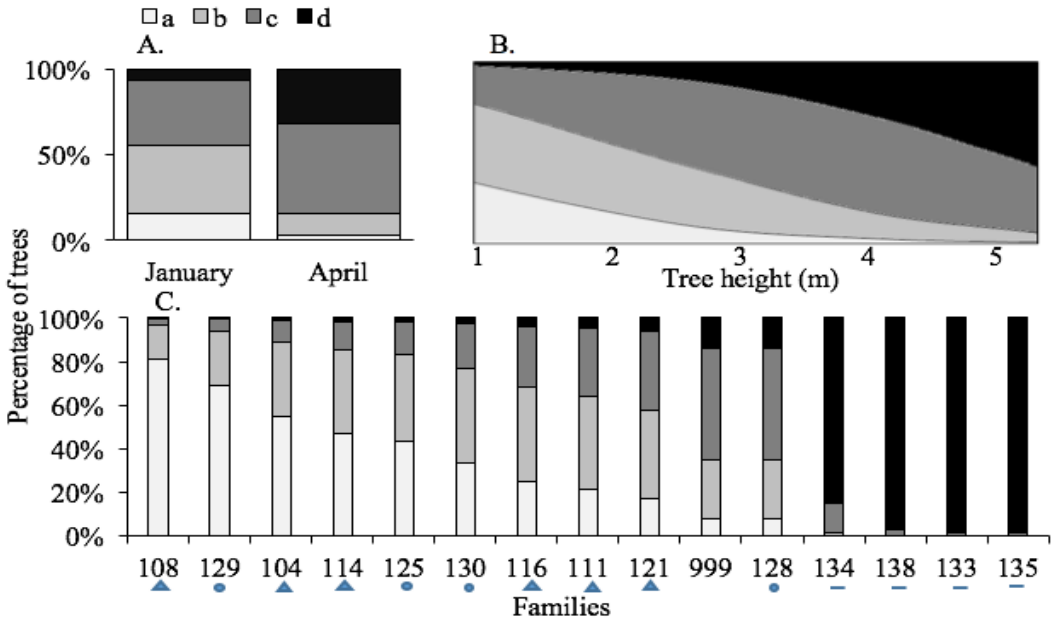



**Figure 4.12** Fixed effects of the final CLMM of the tree grading method (a = no or little chewing (<5%); b = light chewing (5–25% defoliation); c = moderate chewing (26-40% defoliation); d = moderately severe chewing (> 40% defoliation)), showing the effects of time, tree height and family on the chewing damage level of trees: A) time effect; B) tree height effect; C) family effect (families ordered based on the percentage of moderately severe damage). Family provenance = – Southern, ● Bungonia, ▲ Marulan.

*4.4.2.5. Summary of result for family susceptibility to* P. charybdis

Laval abundance and defoliation levels revealed differences in the susceptibility of *E. bosistoana* families to *P. charybdis*. Rankings of family susceptibility to *P. charybdis* were not completely consistent between assessment methods, but generally, the Southern provenance families, 133, 134, 135 and 138, were the most susceptible, followed by family 128 (Bungonia provenance), ranked 5th most susceptible by all methods (Table 4.3). The average rank across all methods was calculated to attain an overall rank of family susceptibility. Family 133, 135, 138, 134 and 128 were again the most susceptible, while family 108, 104 and 114 were the least susceptible. Family 111 and 121 were ranked 1st and 2nd using the pest counting method, but were only found to be moderately susceptible relative to other families based on the defoliation measures used in the other 3 methods. The largest variation in ranking was observed for family 999; ranked as the least attacked based on the pest counting and CDI shoot methods, but was ranked 6th/15 and 8th/15 based on the CDI (tree) and grading methods. This was followed by family 111, which was ranked the most susceptible family using the pest counting method, but 7th or 8th using all other methods.

**Table 4.3** Relative rank (1 = most susceptible to 15 = least susceptible) of *E. bosistoana* and *E. globoidea* (999) families with respect to attack from *P. charybdis* based on four different heath assessment methods.

| Family | Rankings by assessment methods | | | | | |
|---|---|---|---|---|---|---|
| | Pest counting | CDI (tree) | CDI (shoot) | Tree grading | Average ranking | |
| 133 | 3 | 1 | 1 | 2 | 1.75 | 1 |
| 135 | 4 | 3 | 3 | 1 | 2.75 | 2 |
| 138 | 6 | 2 | 2 | 3 | 3.25 | 3 |
| 134 | 7 | 4 | 4 | 4 | 4.75 | 4 |
| 128 | 5 | 5 | 5 | 5 | 5 | 5 |
| 111 | 1 | 7 | 7 | 8 | 5.75 | 6 |
| 121 | 2 | 6 | 8 | 7 | 5.75 | |
| 125 | 10 | 13 | 6 | 11 | 10 | 7 |
| 130 | 8 | 12 | 12 | 10 | 10.5 | 8 |
| 116 | 14 | 10 | 11 | 9 | 11 | 9 |
| 129 | 12 | 9 | 9 | 14 | 11 | |
| 999 | 15 | 8 | 15 | 6 | 11 | |
| 114 | 11 | 11 | 13 | 12 | 11.75 | 10 |
| 104 | 9 | 14 | 14 | 13 | 12.5 | 11 |
| 108 | 13 | 15 | 10 | 15 | 13.25 | 12 |

Family ranks based on the CDI (tree) and tree grading methods were strongly correlated (r=0.914) (Table 4.4). Correlations between the pest counting method and other three methods were only moderate (r = 0.575 to 0.625). Both CDI (tree) and tree grading methods were strongly correlated with the overall average rank (r = 0.936 and 0.901 respectively).

**Table 4.4** Correlations (Pearson's r) of family rank to *P. charybdis* attack between the four health assessment methods and the overall average ranking.

| Methods | Pest count | CDI (tree) | CDI (shoot) | Tree grading | Average rank |
|---|---|---|---|---|---|
| Pest count | | 0.625 | 0.661 | 0.575 | 0.811 |
| CDI (tree) | 0.625 | | 0.764 | 0.914 | 0.936 |
| CDI (shoot) | 0.661 | 0.764 | | 0.689 | 0.882 |
| Tree grading | 0.575 | 0.914 | 0.689 | | 0.901 |
| Average rank | 0.811 | 0.936 | 0.882 | 0.901 | |

### 4.4.3. Susceptibility of *E. bosistoana* families to *O. eucalypti*

The susceptibility of *E. bosistoana* families to *O. eucalypti* was only assessed using the shoot counting method, because the insect produces chewing damage that looks the same as *P. charybdis* damage. Due to the fact that population of *O. eucalypti* was much smaller than *P. charybdis*, as reported in Chapter 2 (Figure 2.4 & 2.8), chewing damage observed was treated as damage caused by *P. charybdis*.

The ranking of family susceptibility to *O. eucalypti* larvae was not consistent over time (Figure 4.13). Larval abundance was highest on family 116 in March 2016, yet no larvae were observed on the family in February 2016. In contrast, larval abundance was higher on families 121, 108 and129 on the other sampling occasions.

Rankings determined based on the abundance of *O. eucalypti* egg batches showed similarities between sampling seasons. Eggs were most commonly found on families 108, 116 and 121 in November 2015 (season 1) and early December 2016 (season 2). No egg batches were found on any trees in family 999 and family 130 across all months, however low numbers of larvae were found on trees in family 130 (Figure 4.14)

The fixed effects part of the final GLMM on *O. eucalypti* abundance indicated that tree height and time (with interaction), and the relative proportion of flush and expanding leaves (without interaction) had a significant effect on larval abundance. The proportion of flush and expanding leaves was positively correlated with larval abundance. In contrast, the relationship between larval abundance and tree height was negative. The random effects part of the model indicated that family had no significant effect on *O. eucalypti* larval abundance. GLMMs also indicated that there was no significant effect of family observed on egg batch abundance.

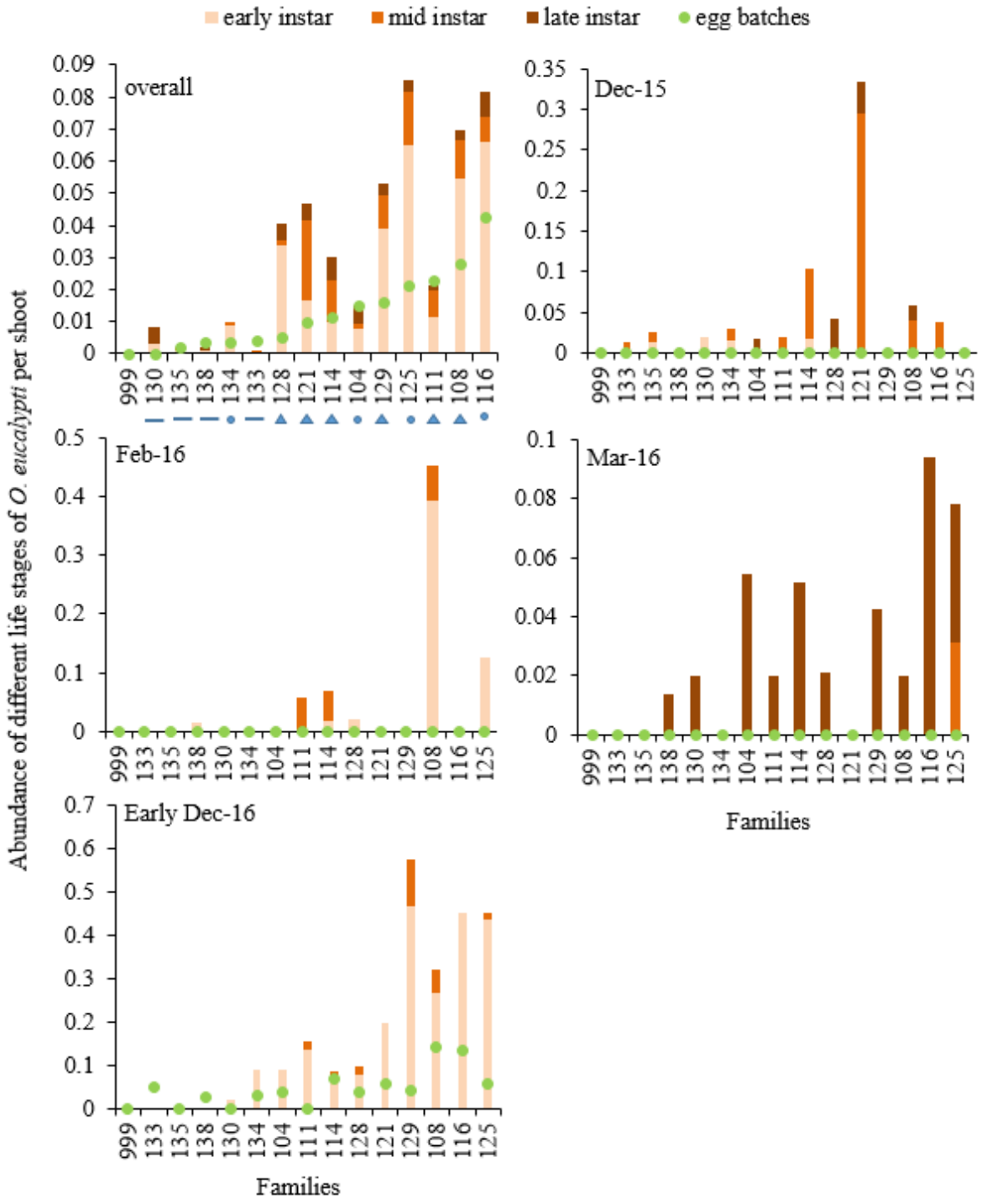



**Figure 4.13** Abundance of different life stages of *O. eucalypti* per shoot in Dec-15, Feb-16, Mar-16 and early Dec-16 and the overall average for these months. Families are ordered based on the overall average egg batches per shoot across the sampling season. Family provenance = – Southern, ● Bungonia, ▲ Marulan.

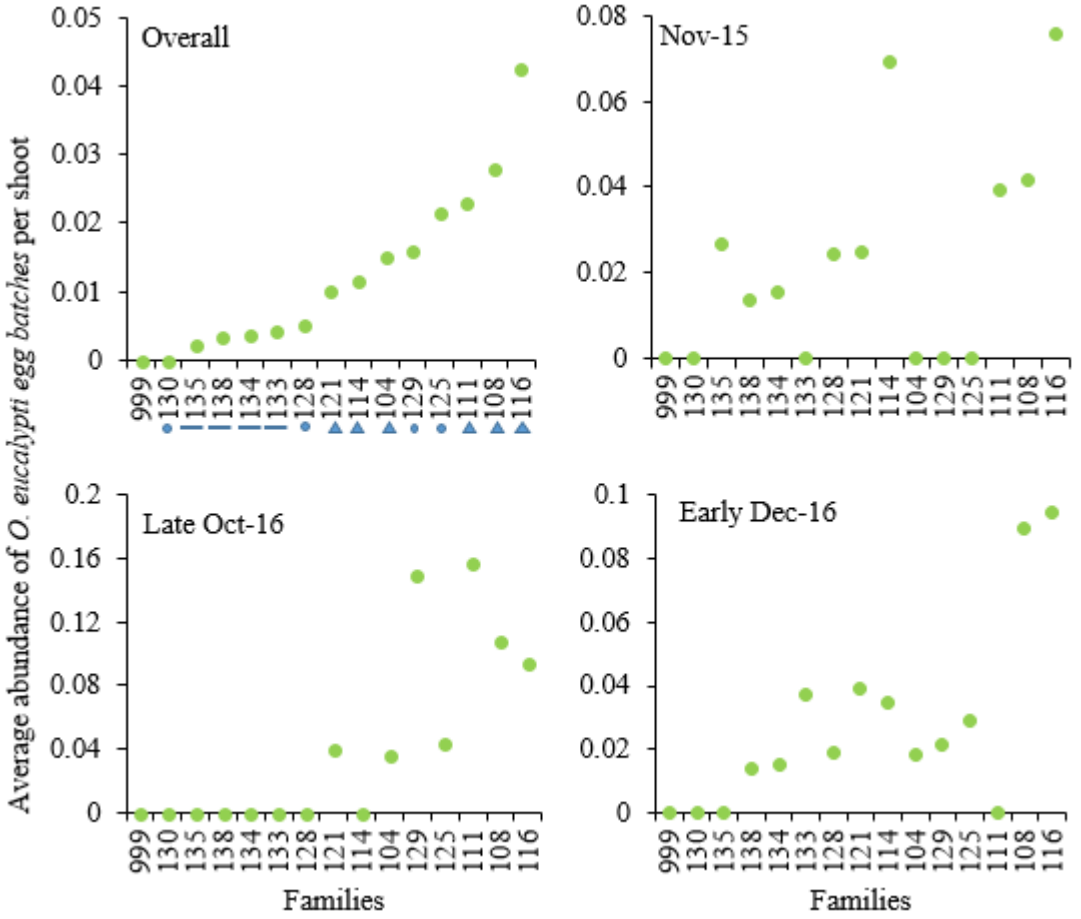



**Figure 4.14** Abundance of *O. eucalypti* egg batch per shoot. Families are ordered based on the average abundance of egg batches per shoot across all sampling occasions. Family provenance = – Southern, ● Bungonia, ▲ Marulan.

### 4.4.4. Susceptibility of *E. bosistoana* families to *S. macropetana*

*4.4.4.1. Pest counting method*

In contrast with *P. charybdis*, trees from the Southern provenance families had less pest loads of *S. macropetana* compared with trees from the Marulan and Bungonia provenances over the experiment period (Figure 4.15). Trees in family 104 (Marulan provenance) had the largest pest load of *S. macropetana* in season 1, while *E. globoidea* supported very small number of *S. macropetana* over the sampling period.

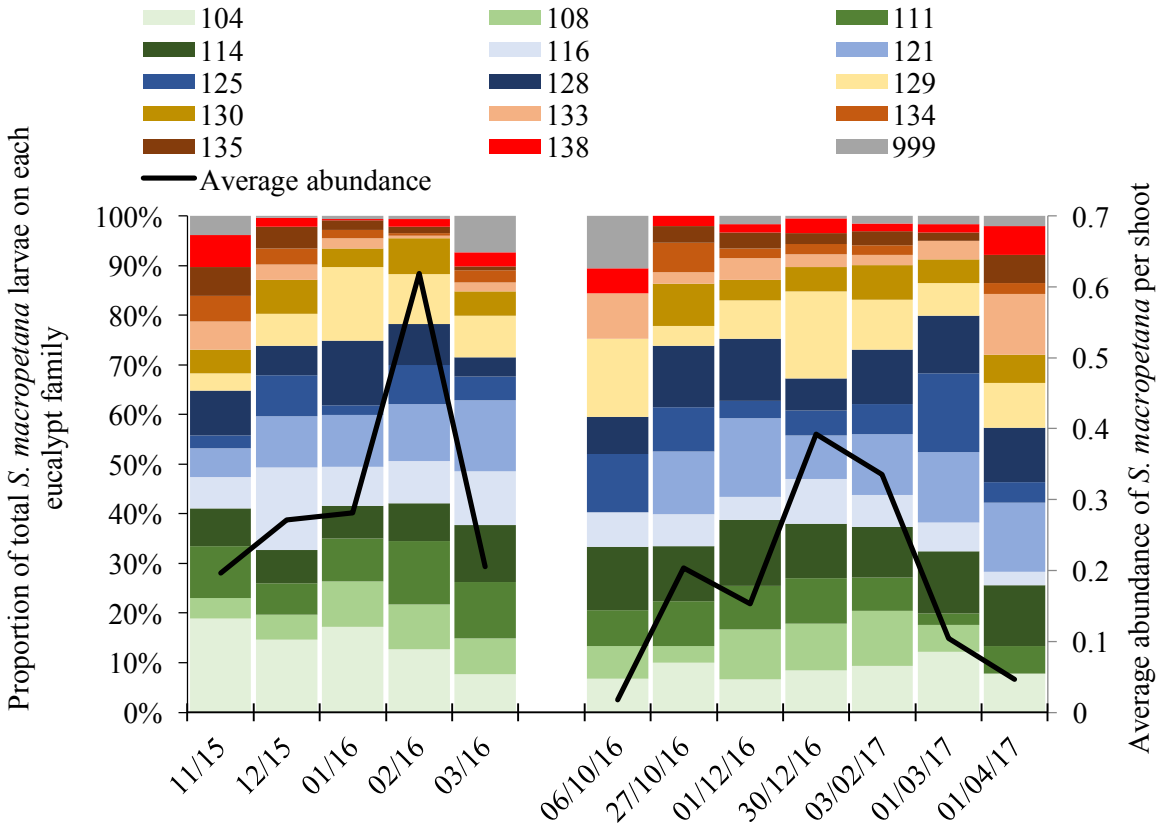



**Figure 4.15** Proportion of total *S. macropetana* larvae counted per sampling occasion that were detected on each eucalypt family. Black solid line represents the total average abundance of *S. macropetana* larvae per shoot across all families combined over the experiment period.

The fixed part of the best-fitting model of pest abundance on *S. macropetana* (Table 4.2) indicated that abundance of the leafroller and tree height had a signficant negative correlation, while the proportion of flush and expanding leaves (the soft leaves) had a signficant positive correlation with the leafroller abundance.

The random effects part of the best-fitting model indicated a significant family effect that varied slightly between sampling occasions. From the random effects part of the model with family effect without the interaction of time, we could see the overall effect of family across different sampling events: *Eucalyptus globoidea* (999) and the Southern provenance families (134, 133, 138 and 135) had the lowest *S. macropetana* abudance, while all the families from the Marulan provenance (121, 111, 114, 104, 106 and 108) had the highest leafroller abundance (Figure 4.16a). Families from the Bungonia provenance had less pest abundance than the Marulan provenance. Rank of families varied between sampling occasions, but except the Southern provenance families and 999, other families were generally had above than average abundance of the leafroller (Figure 4.16b-e).

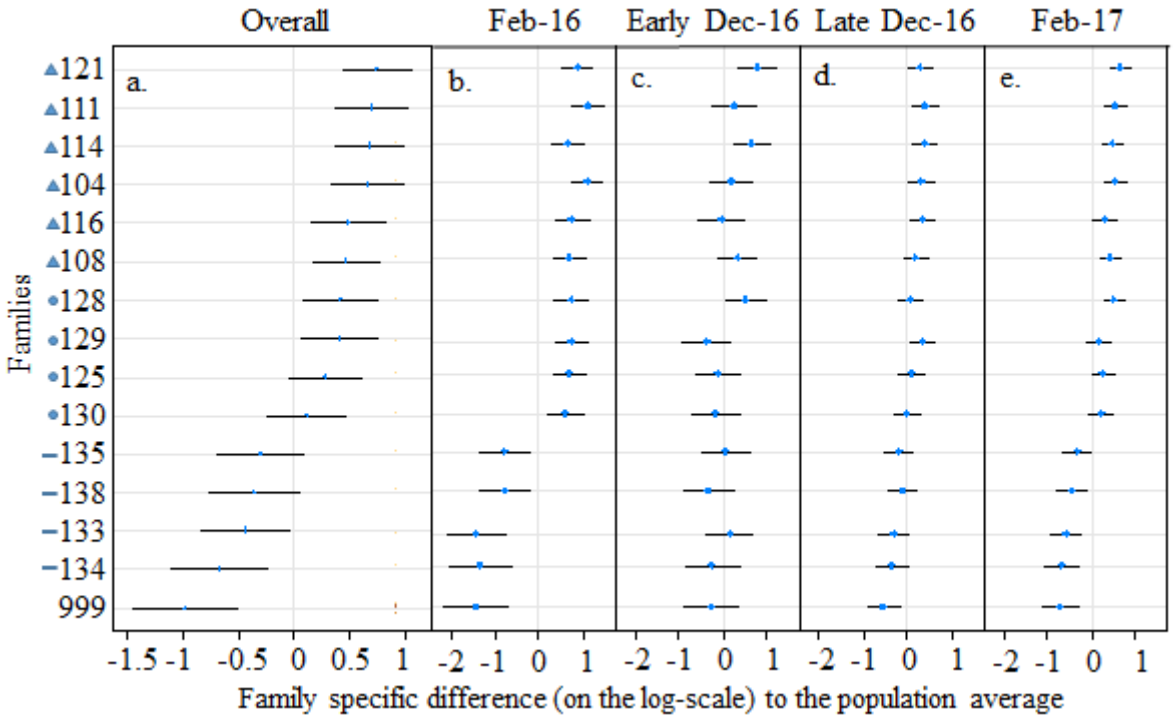



**Figure 4.16** Random effects of family. Difference (± 95% Cl) of family-specific parameter values from the overall average of the *S. macropetana* abundance (shown as 0) predicted by GLMMs constructed for pest counting method: a) over all sampling occasions (model with family random effect without interaction with time); b - e) for each separate sampling occasion (model with family random effect as family × time). Families are ordered based on the predicted family specific difference to the population average (from top to bottom was the family with the most pest abundance to the family with the least pest abundance). Positive values = greater pest load than the overall average vs. negative values = less pest load than the overall average. Family provenance = – Southern, ● Bungonia, ▲ Marulan.

*4.4.4.2. CDI (tree) method*

Leaf roller damage recorded using the CDI (tree) method was much lower than *P. charybdis* chewing damage, such that no trees were observed to have sustained leaf roller damage to > 7% of the crown. Similar to the results of the pest counting method (Figure 4.16), families from the Southern provenance and family 999 were the least damaged by *S. macropetana* relative to other families. Family 104 was the most damaged family, followed by 129 and 108 (Figure 4.17). Average damage to trees in family 104 was 2.56%. The most damaged families were from Marulan provenance, except family 129 (Bungonia provenance). The best fitting LMM (Table 4.2) predicted that only tree height had a significant (negative) effect on damage by *S. macropetana*. Effects of all other factors, including family, were not significant.

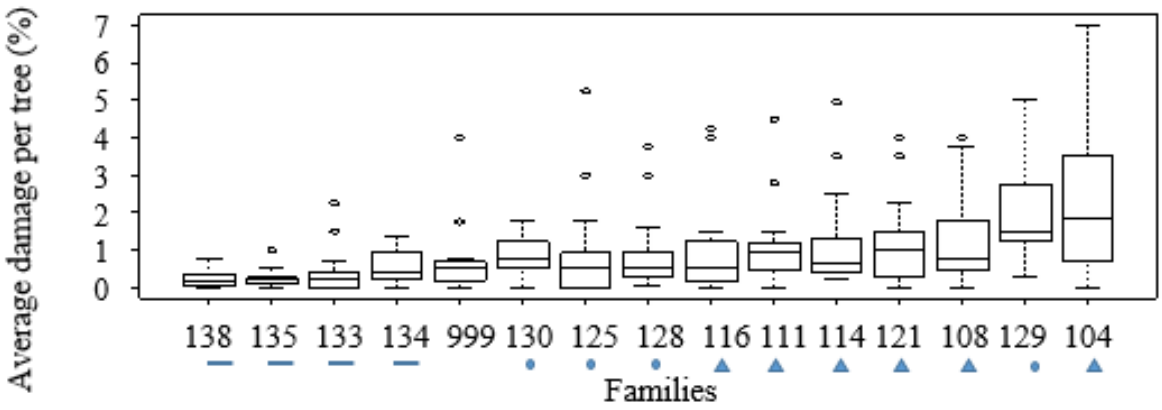



**Figure 4.17** Average percentage of crown damaged per tree by *S. macropetana* for each eucalypt family (from least to most damaged) in December 2015. Family provenance = – Southern, ● Bungonia, ▲ Marulan.

### 4.4.5. Susceptibility of *E. bosistoana* families to *Ph. froggatti*

*4.4.5.1. Pest counting method*

In season 1, families 104, 121 and 128 were generally heavily attacked by *Ph. froggatti,* but families 129, 134 and 108 were predominant in season 2 (Figure 4.18). Family 133 and 135 (Southern provenance) and family 999 (*E. globoidea*), were the least damaged family over the experiment period. The final LMM on pest counting on *Ph. froggatti* (Table 4.2) indicated that only time and tree height had significant effects on *Ph. froggatti* abundance. Abundance was negatively correlated with tree height. The effect of family on *Ph. froggatti* abundance was not significant .

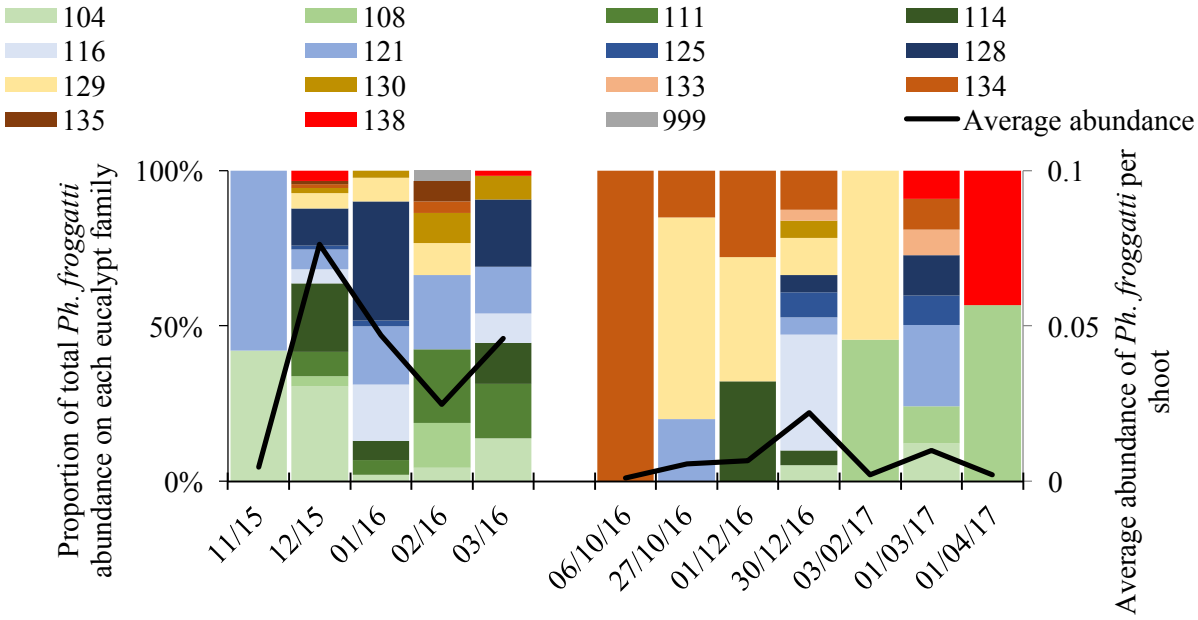



**Figure 4.18** Proportion of total *Ph. froggatti* abundance counted per sampling occasion that were detected on each eucalypt family. Black solid line represents the total average abundance of *Ph. froggatti* per shoot across all families combined over the experiment period.

*3.4.5.2. CDI (tree) method*

Most trees sustained mining damage to 0%-20% mining damage of lower crown foliage, while only 3 trees had a CDI score of >30%. Using the CDI (tree) method to assess susceptibility to *Ph. froggatti*, the most damaged family was again family 104 (mean damage per shoot = 11%) followed by family 129 (10%). Family 999 suffered the least average damage, well below 1% per shoot, followed by family 133 at 1.27% (Figure 4.19).

The fixed effects part of the final model (Table 4.2) showed that family and the relative proportion of mature leaves had significant effects on incidence and severity of mining damage. A positive correlation was found between the damage level and the relative proportion of mature leaves. Families with higher than average damage were 104, 128, 129, 114, 130 and 116 which were all from the Bungonia and Marulan provenances (Figure 4.20). The *Monocalyptus* family 999 suffered the least damage. Families predicted to have lower than average damage included all Southern provenance families.

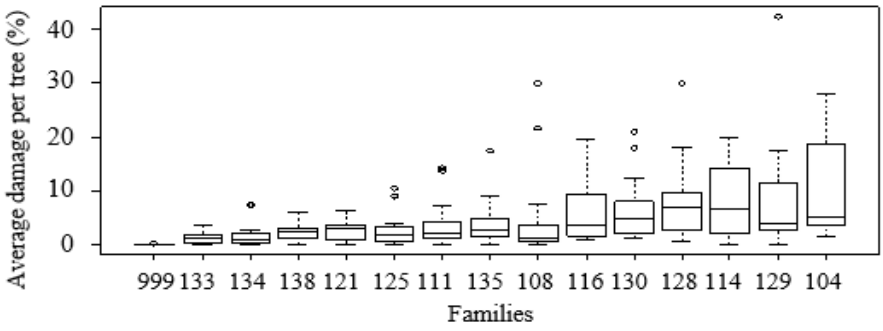



**Figure 4.19** Average percentage of crown damaged per tree by *Ph. froggatti* for each eucalypt family (from least to most damaged) in December 2015. Family provenance = – Southern, ● Bungonia, ▲ Marulan.

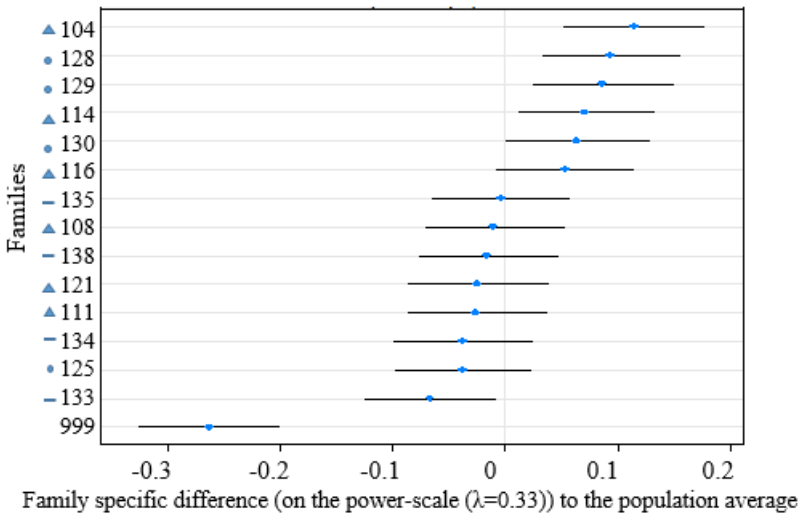



**Figure 4.20** Random effects of family. Difference (± 95% Cl) of family-specific parameter values from the overall average mining damage per tree (shown as 0) predicted by the final LMM constructed for CDI (tree) on *Ph. froggatti*. Families are ordered from the most defoliated (top) to the least defoliated. Positive values = greater damage than the overall average vs. negative values = less damage than the overall average. Family provenance = – Southern, ● Bungonia, ▲ Marulan.

*4.4.5.3. CDI (shoot) method*

Generally, average mining damage per shoot was very low, however, some individuals exhibited >40% damage in some months (See circles in Figure 4.21). Most of these were located in the lower crown and damage observed was from previous year(s). Family 104 had the greatest mining damage per shoot overall (8.00% and 4.05% for Nov-15 and Dec-15 respectively) but also had the largest variation in damage between trees relative to other families in November and December 2015 (Figure 4.21). It was followed by family 130 and 114 in November 2015, and by family 129 and again 114 in December 2015. Family 999 and 133 sustained the least mining damage ranging from 0.02% to 0.72%.

The fixed part of the final GLMM model (Table 4.2), indicated a significant negative correlation between tree height and the probability of mining damage being present. The probability of mining damage being present varied significantly between at least two months. There was a significant positive correlation between the relative proportion of mature leaves and the probability of mining damage being present.

The random part of the final model shown that family had a significant effect on the presence or absence of mining damage, and the effect was consistent across months. Families predicted to have the greatest probability of damage were from the Bungonia and Marulan provenances (Figure 4.22). Family 129 and 104 were predicted to be more likely to have mining damage. The family found to have the least probability of damage was the *Monocalyptus* family 999. Families from the Southern provenance all had less than average probability of mining damage, although 135 was very close to the population average.

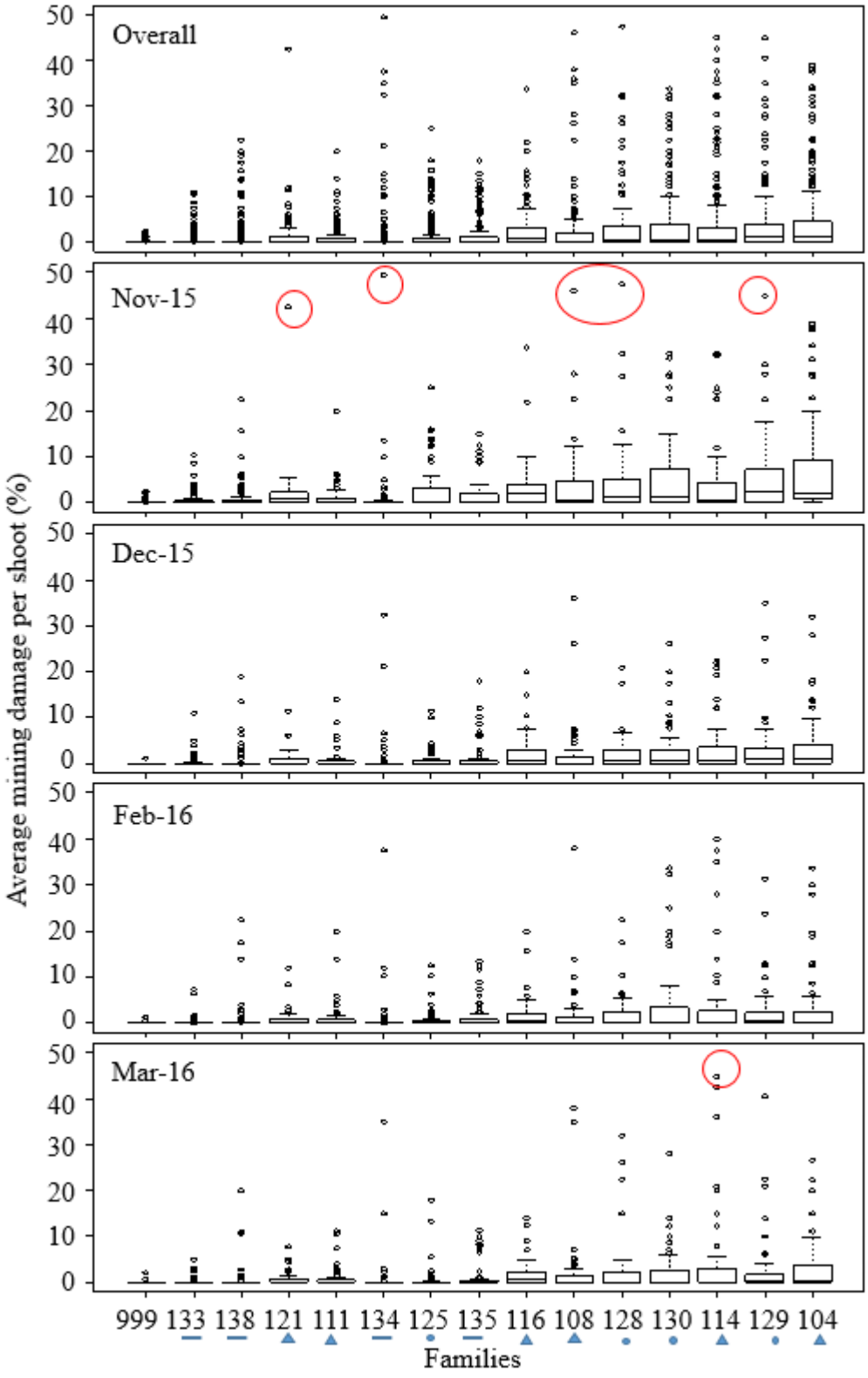



**Figure 4.21** Percentage of mining damage (incidence x severity) per shoot for trees of different families overall all months and in each month separately Families are ordered from the least to most damage averaged over all sampling occasions. Family provenance = – Southern, ● Bungonia, ▲ Marulan. Red circles show the individual trees with over 40% damage.

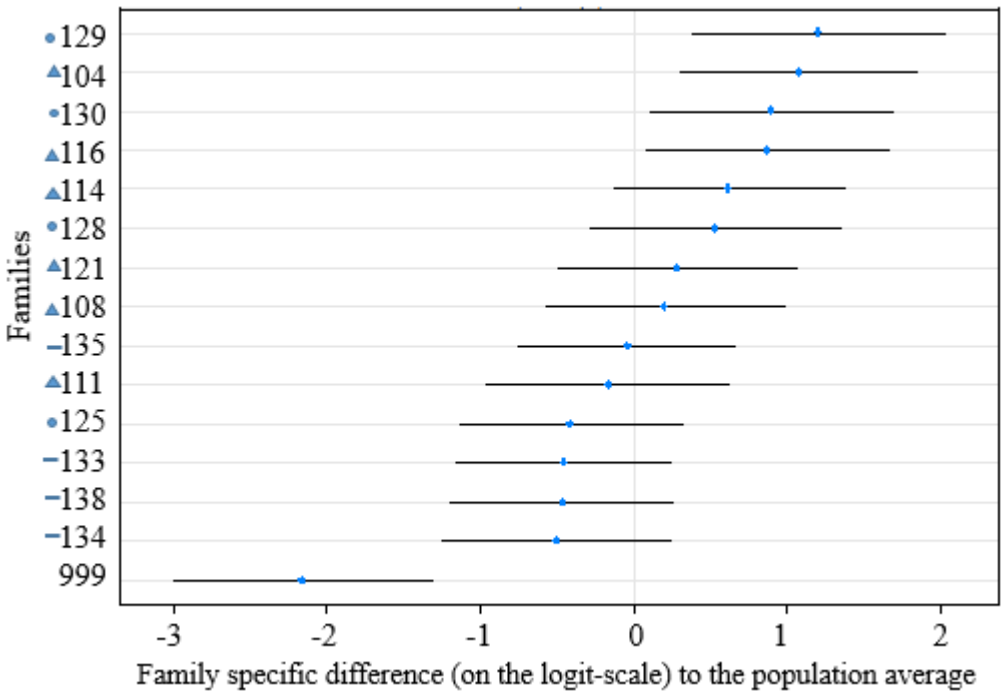



**Figure 4.22** Random effects of family - difference (± 95% CI) of family-specific parameter values from the overall average probability of *Ph. froggatti* mining damage being present (shown as 0) predicted by the final GLMM constructed for CDI (shoot). Families was ordered based on the family specific difference to the population average (from top to bottom was the family most likely to have mining damage to the family least likely to have mining damage). Positive values = higher probability than the overall average vs. negative values = lower probability than the overall average. Family provenance = – Southern, ● Bungonia, ▲ Marulan.

*4.4.5.4. Summary of results for family susceptibility to* Ph. froggatti

Rankings of family susceptibility to *Ph. froggatti* using the pest counting, CDI (tree) and CDI (shoot) method were not fully consistent (Table 4.5). Results from two of the CDI methods were similar, and supported by a significant strong ($P<0.01$, $r=0.87$) correlation between family rankings for susceptibility. However, significant differences were not found between families using the pest counting method.

**Table 4.5** Rank of family susceptibility to *Ph. froggatti* based on different assessment methods, and the overall average rank from all three methods. Families are ranked from 1 (most susceptible) to 15 (least susceptible).

| Family | Rank from different assessment methods | | | |
|---|---|---|---|---|
| | CDI (tree) | CDI (shoot) | Average rank | |
| 104 | 1 | 2 | 1.5 | 1 |
| 129 | 3 | 1 | 2 | 2 |
| 128 | 2 | 6 | 4 | 3 |
| 130 | 5 | 3 | 4 | |
| 114 | 4 | 5 | 4.5 | 4 |
| 116 | 6 | 4 | 5 | 5 |
| 108 | 8 | 8 | 8 | 6 |
| 135 | 7 | 9 | 8 | |
| 121 | 10 | 7 | 8.5 | 7 |
| 111 | 11 | 10 | 10.5 | 8 |
| 138 | 9 | 13 | 11 | 9 |
| 125 | 13 | 11 | 12 | 10 |
| 133 | 14 | 12 | 13 | 11 |
| 134 | 12 | 14 | 13 | |
| 999 | 15 | 15 | 15 | 12 |

### 4.4.6. Summary of family susceptibility to four key insect defoliators

Although pest tolerance (ability to withstand damage) was not measured directly, tree height combined with observed insect load and defoliation levels gives a good indication of tolerance (Figure 4.23). Family 111, 121 and 128 should be avoided for future planting because they were slower growing (shorter) and most susceptible (based on pest load and damage observed) to the most damaging eucalypt defoliator in New Zealand, *P. charybdis*. In contrast, family 125 was both faster growing and less susceptible. Families 138, 135, 133 and 134 may also have high tolerance to *P. charybdis as*, although relatively susceptible, they were taller than most other families, suggesting fast growth even in the presence of defoliation. If leafrollers like *S. macropetana* are of particular concern, families from the Marulan provenance could be eliminated as they were both highly susceptible to leafroller attack and slower growing. In terms of *Ph. froggatti*, families 129, 128,130 and 104 should be eliminated, whereas the Southern provenance families, the *Monocalyptus* family and family 125, should be maintained in the breeding programme.

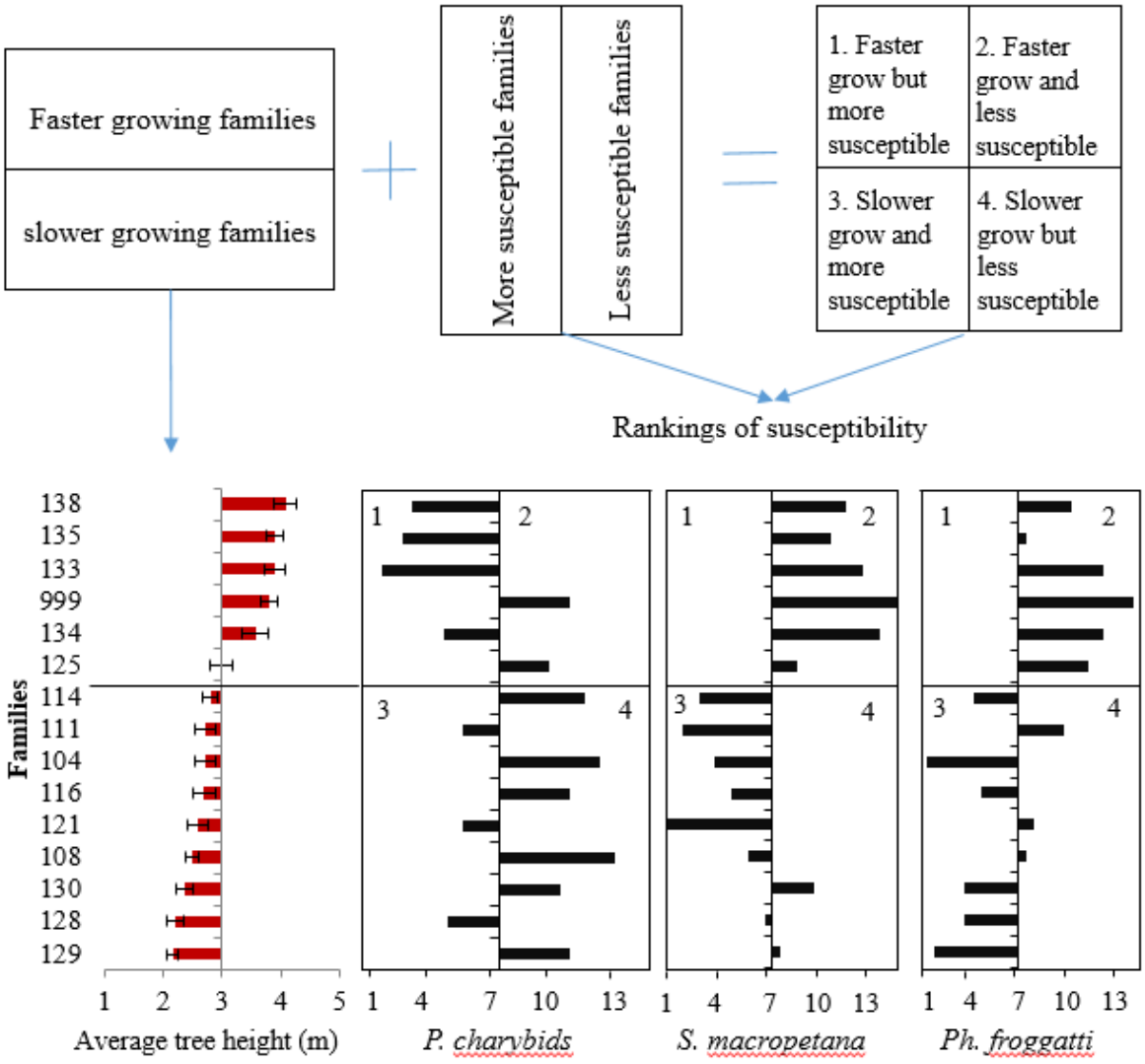



**Figure 4.23** Selection criteria for choosing suitable families for future breeding trials based on tree growth and susceptibility to different guilds of insect pests (*P. charybdis* = chewer; *S. macropetana* = leafroller; *Ph. froggatii* = leafminer). Faster growing families are defined as those of above average tree height and slower growing families as those of below average tree height. Families ranked between 1st/15 and 7.5th/15 based on insect load and levels of defoliation were defined as more susceptible and families ranked between 7.5th/15 and 15th/15 were defined as less susceptible.

## 4.5. Discussion

### 4.5.1. Family variation in insect susceptibility

With respect to the first objective of this chapter, significant variation in susceptibility to all of the insect defoliators examined, except *O. eucalypti*, were observed between 14 *E. bosistoana* families and 1 *E. globoidea* family. This was indicated by the significant effects of family on pest abundance and/or defoliation of whole trees and selected shoots. Overall, family 125 (Bungonia provenance) and 999 (*E. globoidea*) were consistently less susceptible to all examined pests (except *O. eucalypti*) and had average or above-average growth. In contrast, family 128 (Bungonia provenance) exhibited consistently higher susceptibility. Interestingly, the Southern provenance families were most heavily defoliated but showed higher tolerance

(sustained more damage but had faster growth) to chewing damage relative to other families, and were relatively more resistant to *S. macropetana* and *Ph. froggatii*.

The significant variation in *P. charybdis* susceptibility is consistent with previous studies. Provenance and genotype within individual *Eucalyptus* species have been found to affect host preference of a number of paropsine beetles. For example, significant differences in *P. charybdis* damage were found between and within several *Eucalyptus* species in New Zealand (Hathaway and King 1986). *Paropsisterna atomaria* larvae were found to have significant feeding preferences on selected *E. grandis* genotypes in south-eastern Australia, and larval development rates when reared on foliage from different genotypes were also significantly different (Johns et al. 2004). Large family differences in defoliation caused by *Paropsisterna bimaculata* have been found for *E. regnans* and *E. nitens* (Raymond 1995). However, paropsine beetles have also been observed to switch to feeding on resistant species and genotypes when food is limited. For example, *Paropsisterna* nr. *gloriosa* Blackburn were found to have a feeding preference for *E. parvula*, but the highly resistant *E. pulvernulenta* and *E. cordata* were found to be accepted by the beetles in plantations with the highest levels of damage, due to an "overflow" of the pests from the preferred hosts (Horgan 2011). This highlights the needs to select for general insect tolerance in future breeding, rather than specific resistance. Host preference could also vary between eucalypt beetle life stages. Oviposition, larvae abundance and defoliation by *Pst. agricola* were found to be significantly different between *E. globulus* families, but no significant effect of genotype was found on adult beetles distribution in the field (Rapley et al. 2005), likely due to the mobility of adult beetles. Within-species variation in susceptibility to *S. macropetana* has also been found in the few previous studies (e.g. Hathaway and King 1986, Mauchline et al. 2001), and provenance variation in *Ph. froggatti* damage has been observed on *E. globulus* in Australia (Farrow et al. 1994, Floyd et al. 2002).

Variations in nutritional, chemical, physical and phenological properties of foliage between and within eucalypts species (Ohmart and Edwards 1991, Ohmart 1991, Steinbauer 2001, Henery et al. 2008) provide the basis for selecting insect resistant or tolerant species and genotypes, and for eliminating the most susceptible species and genotypes with otherwise desirable growth and wood properties traits. Physical properties, such as waxes and sclerophylly, can inhibit oviposition and larval feeding (Ohmart and Edwards 1991, Horgan 2011). Foliar chemistry also affected insect behaviour, and some of the chemical compounds in eucalypt foliage have been linked to insect tolerance (Li 1994). For insects such as paropsine beetles where the early instar larvae depend on soft leaves, tree phenology may be more important to host preference. Soft leaves, and the factors affecting leaf toughness, such as the timing of foliage appearance in the field are suggested to be critical factors to host preference for paropsine beetles (Steinbauer 2001, Johns et al. 2004, Howlett 2005). In the current study, leaf age significantly affected pest abundance and defoliation caused by all examined pests, but as the leaf type factors were fixed effects in the statistical models (Table 4.2) and family random effects, it indicated that other foliar properties besides leaf age also contributed to the insect host preference. The Southern families had lower proportions of flush and expanding leaves relative to other *E. bosistoana* families for most of the experimental period, but were still the most susceptible families based on pest load and defoliation. This reflects that insect susceptibility of families is likely the result of complex interactions between the nutritional, chemical, physical and phenological properties of the host species. Thus, it is difficult to depend on any single trait to identify insect resistant or tolerant genotypes. However, the visually estimation of leaf age may not have been accurate enough for the purpose to identify host suitability, and a better indicator for leaf toughness, such as specific leaf weight (Steinbauer 2001), may be more useful to examine its effects on pest host preferences in future

studies. Also, chemical and physical leaf properties were not examined in this study, and should be tested in durable eucalypts in the future.

Due to the difference in insect biology and the complexity of insect-host interactions, *Eucalyptus* family susceptibility to different insects can be expected to vary, as was found here. Integrated pest management in plantation forestry generally focuses on the pests that can cause the most severe production loss, but their biology and interactions with the hosts may differ markedly from that of other pests which may also have the potential to cause damage under certain conditions. Consequently, it is essential to understand the specific preferences of pest species from different feeding guilds when selecting suitable species, families and genotypes for future planting and breeding stock. Results of this study indicated that the family effect on tree susceptibility was not significant for all examined defoliators. Tree improvement that eliminates the most susceptible families or maintains the most tolerant families will not benefit the management of *O. eucalypti* as no significant variation was observed between families to select on, but it would possibly reduce the risk of outbreaks of *P. charybdis* (indicated by all assessment methods), *S. macropetana* (indicated by the pest counting method) and *Ph. froggatti* (indicated by the CDI (tree and shoot) methods). It is not surprising *Eucalyptus* families examined in this study were all utilised to a similar degree by *O. eucalypti* as it has been shown that *O. eucalypti* can feed on other tree species such as the pepper tree *Schinus molle* Sharell, common apple trees *Malus domestica* Miller, and birch trees (Meyer-Rochow 1986).

Provenance was a good indicator of insect tolerance. As noted above, all families from the Southern provenance sustained more chewing damage and *P. charybdis* abundance (but continued to grow) and less *S. macropetana* and *Ph. froggatti* damage. Although the families within the Bungonia and Marulan provenances responded differently to insect attack (especially the Bungonia provenance with one family (125) appearing relatively resistant to the examined defoliators), provenance may still be a useful criterion to inform the initial selection for breeding programmes or provenances within programmes could be compared for insect tolerance rather than all genotypes, reducing assessment time in the initial stages.

Besides the effect of family, tree height and leaf age also exhibited significant effects on pest abundance and insect damage level, but the effect differed between pest species. Tree height was positively correlated with susceptibility to *P. charybdis*, but a negatively correlated to susceptibility to the other three pests. The former contrasts with previous studies, in which susceptibility to paropsine beetles was negatively correlated with tree height and the slower growing genotypes were more susceptible to leaf beetle damage (Ohmart et al. 1984, Raymond 1995, Rapley et al. 2005). One hypothesis for the negative correlation between susceptibility and tree height/tree size is that faster growing trees have more adult foliage and juvenile leaves switch earlier to adult foliage (Jordan et al. 2000). However, it has also been found that plant growth can be significantly negatively correlated with plant defence and subsequently positively correlated with herbivore damage (Coley 1988). The effect of tree height on insect susceptibility of trees in this study was consistent with previous studies (Hathaway and King 1986, Mauchline et al. 1999). *Paropsis charybdis, O. eucalypti* and *S. macropetana* preferred flush and expanding leaves as expected, while *Ph. froggatti* preferred mature leaves presumably because they are more suitable for mining than leaves that are still expanding.

### 4.5.2. Comparing the *Monocalyptus* family to *E. bosistoana* families

The *E. globoidea* family 999 was planted as part of all NZDFI breeding trials of different species across many different sites as a control for understanding site effects. For this study it

provided a useful comparison as a representative of the subgenus *Monocalyptus*. Overall, *E. globoidea* was relatively less susceptible to all examined defoliators compared to the *E. bosistoana* families. It was ranked as the least susceptible family overall to *S. macropetana* and *Ph. froggatti*, and 10th/15 to *P. charybdis*. Although there are some counter examples (e.g. Mauchline et al. (2001) on *S. macropetana*), these results were consistent with previous studies that have found the subgenus *Monocalyptus* is less susceptible to insect pests than the subgenus *Symphyomyrtus* (Noble 1989, Stone et al. 1998). This may result from *Symphyomyrtus* species tending to have higher total (Millner and Kemp 2012) and available (Wallis et al. 2010) foliar nitrogen than *Monocalyptus* species. However, as large variation in insect susceptibility is also observed within *Symphyomyrtus* (e.g. Steven (1973)) and some *E. bosistoana* families did perform better than the one *Monocalpytus* representative in this study, *Symphyomyrtus* should not be automatically excluded from breeding trials based on the general impression that they are inherently more susceptible to insect damage. Currently, symphyomyrts represent the majority of commercial eucalypts due to their relatively rapid early growth (Harwood 2011) and desirable wood properties (e.g. Bush and Walker (2011)). Although monocalypts have been observed to be less insect susceptible in general, evidence suggests that they have their own specialist herbivores (Li 1994). For example, as Ash species from the subgenus *Monocalyptus* are the natural hosts of *P. bimaculata*, the monocalpyts *E. regnans* and *E. delegatensis* failed to establish in Tasmanian plantations due to the devastating attack by the beetles at the time of canopy closure (Harwood 2011). Furthermore, high degrees of variation in insect susceptibility have also been observed within monocalypts (Morrow 1977). Also, many symphyomyrts are known to have greater durability of heartwood (Bush and Walker 2011) and thus be more suitable in the context of establishing a durable eucalypt industry. Consequently, selecting for insect resistant or tolerant species and genotypes for future breeding should examine the variation between species, provenances and even families of both subgenera.

### 4.5.3. Comparison of field assessment methods

The four methods used to assess family susceptibility to *P. charybdis* produced similar results. However, there were some slight differences. Particularly, family rankings from the tree grading and pest counting methods were only moderately correlated. Results from the methods based on damage level were more consistent with each other compared with the pest counting method. This may be because damage observed can include accumulated damage from previous seasons, while pest counting is a measure of one moment in time. For some pest species, if only targeting a specific life stage, counting pest abundance could miss information on host preferences of other life stages. For example, if only targeting *P. charybdis* larvae, information on preference of adults feeding might be missed. Also, for insects with long adult life spans such as *P. charybdis*, oviposition can occur over many months, and several sampling occasions during this time would be required to accurately determine host preferences, as the results here indicate there may also be interactions with tree phenology which may vary over the egg laying period. It was unfortunate that in this study, the oviposition preference could not be identified for *P. charybdis* because egg abundance was very low and the sampling frequency may not have been sufficient to detect the peak laying period. Also, surveys in season 1 might not have started early enough to catch the peak abundance. However, while results of the two CDI methods were consistent for *P. charybids*, the pest counting method may be more suitable for *S. macropetana*, because old *S. macropetana* damage can be easily confused with damage caused by wind or other insects. While the two CDI methods (tree and shoot) detected significant differences between families for susceptibility to *Ph. froggatti*, the pest counting assessment did not. This may be because the abundance of current season *Ph. froggatti* larvae

was too low for any differences to be detected, whereas the mining damage observed was higher as it included damage from previous years.

The pest counting method differs from the other assessment methods, because it measures pest load rather than damage and is therefore only useful if counts can be used to predict damage. The advantage of the pest counting method is, however, that its quantitative nature makes results easier to compare between studies, sites, or years and there is likely to be less variation between multiple observers than when methods that rely on visual estimates are used. The disadvantages are that counting is more time-consuming and any variation between different species or genotypes may be missed if pest abundance is very low. The CDI (tree) and tree grading methods are potentially faster, but results may not be comparable when multiple assessors are used, and the methods are difficult to apply for larger trees. The CDI (shoot) method may be more quantitative than the CDI (tree) method because estimating defoliation is easier at a smaller scale (shoot) than a larger scale (tree), and the shoot method can be easily applied to larger trees. However, it is more time-consuming. Data analysis and interpretation of the CDI (tree) assessment is more straight forward (using LMMs other than GLMM or CLMM) than other methods. Consequently, in respect to the second objective of the chapter, the most effective and efficient assessment method to assess insect susceptibility in general is the CDI (tree) method, but the pest counting method may be more useful to address questions of a quantitative nature.

## 4.6. Conclusion

While family was not found to significantly affect the abundance of *O. eucalypti*, both family and provenance explained variation in susceptibility to *P. charybdis*, *S. macropetana* and *Ph. froggatti*. Although the Southern provenance families (133, 134, 135 and 138) were the most susceptible to *P. charybdis*, these families were also taller on average. In contrast, the Southern provenance families were less susceptible to *S. macropetana* and *Ph. froggatti*, although families 125 (Bungonia) and 111 and 121 (Marulan) performed better than family 135 with respect to *Ph. froggatti* infestation. *Strepsicrates macropetana* showed a clear preference for families from the Marulan provenance. The monocalypt *E. globoidea* was less susceptible to all four defoliators relative to most of the *E. bosistoana* families.

Tree size (indicated by tree height in this study) and leaf type (indicated by the relative proportions of flush, expanding leaves and mature leaves) were found to affect susceptibility to insects, but the effects varied depending on the insect species. *Paropsis charybdis* damage and abundance had a positive correlation with tree height, which may indicate that *P. charybdis* prefers more vigorous trees, although no direct measure of this was made. In contrast, abundance of the other three defoliator species was negatively correlated with tree height, indicating these species may prefer suppressed or slow growing trees. Although *Ph. froggatti* damage was (as expected) positively correlated with the relative proportion of mature leaves, abundance and/or damage from all other species was positive correlated with the proportion of flush and expanding leaves. This preference for soft young leaves may be partly explained by the reduced toughness and higher food quality of these leaves relative to scelerotised mature leaves.

Results from this study indicate that selecting faster growing families or undertaking management practises that promote tree vigour, such as applying fertilizer and good site-species matching, could reduce the defoliation risk from *O. eucalypti*, *S. macropetana* and *Ph.*

*froggatti*. In contrast, *P. charybdis* was shown to prefer the faster growing families, however, it is possible that rapid growth may compensate for some of the losses caused by insect damage. This may allow trees to tolerate relatively high levels of damage, but will require further investigation. One *E. bosistoana* family (125) showed above average tree height and relatively low susceptibility to *P. charybdis* and other defoliators and it is therefore recommended this family be maintained in the breeding programme.

The comparison of methods here shows that the CDI (tree) method is the most effective and practical for use in a young plantation setting and should be used to assess insect susceptibility of all 40 *E. bosistoana* families and other species within the NZDFI breeding programme. However, CDI (tree) is not suitable for larger trees since it is difficult to visually examine the upper crown, therefore, the CDI (shoot) or pest counting methods may be better choices for assessing closed canopy plantations. While the pest counting method is more time consuming and may require multiple assessments per season to account for insects with long egg laying periods, its quantitative nature will benefit scientific research or breeding programmes as it allows direct comparisons of results between sites and years.

# CHAPTER 5 INCIDENCE OF AND DEFOLIATION BY A NEWLY INTRODUCED PEST, *PAROPSISTERNA VARIICOLLIS* (COLEOPTERA: CHRYSOMELIDAE), ON ELEVEN DURABLE *EUCALYPTUS* SPECIES IN HAWKE'S BAY, NEW ZEALAND

In March 2016, *Pst. variicollis* (Chapuis) (*Eucalyptus* variegated beetle, EVB) (Coleoptera: Chrysomelidae) was detected in New Zealand for the first time during routine Forest Biosecurity Surveillance at Te Pohue in Hawke's Bay, North Island (Rogan 2016). A biosecurity response was initiated by the Ministry for Primary Industries including delimitation and surveillance (Rogan 2016) while response options were assessed. The response was closed in autumn 2017 following confirmation that the beetle was widespread across parts of the Hawke's Bay. The pest potential of *Pst. variicollis* is not yet clear, but it is likely to affect the suite of drought- and frost-tolerant eucalypts being assessed by the New Zealand Dryland Forests Initiative (NZDFI) to develop a naturally ground-durable eucalypt timber resource in dryland regions of New Zealand (Millen 2009, Apiolaza et al. 2011b), including the Hawke's Bay.

*Pst. variicollis* is closely related to the known pest *P. charybdis*, and observed to have characteristics that faciliate invasiveness and pest potential. As within *E. bosistoana* variation in insect susceptibility and tolerance to *P. charybdis* was observed, it was considered appropriate to assess *Pst. variicollis* host preferences and impacts on dryland eucalypts as part of this thesis. The objective of this chapter is to assess the initial incidence and defoliation caused by *Pst. variicollis* within durable eucalypt species trials already established close to the initial detection site to understand the risk it poses to the new suite of dryland eucalypts. The methods to assess *Pst. variicollis* host preference and defoliation damage levels of different *Eucalyptus* species (including *E. bosistoana*) was developed from the methods used in assessing the difference of *E. bosisotana* families in insect susceptibility, which was described in Chapter 4 (section 4.3.2). Results from this study will build into understanding the risks faced in establishing new suite of dyland eucalypts and the variation in insect susceptibility between and within durable eucalpyt species.

## 5.1. Introduction

### 5.1.1. Appearance, distribution and phenology

*Pst. variicollis* was formerly named *Chrysophtharta variicollis* before the genus *Paropsisterna* Motschulsky was expanded to include *Chrysophtharta* Weise (Reid 2006, Reid and De Little 2013). Most literature still refers to the species by the former name. Eggs of *Pst. variicollis* are pale yellow and laid in batches on young foliage (Figure 5.1a & b.). Individual eggs are loosely arranged when compared with eggs of *P. charybdis* (Figure 5.1.c. & d.). First instar larvae are tiny with many small black spots, a black head and black terminal segment (Figure 5.1e.). Second and third instars are yellow with a black head and abdominal apex (Tan et al. 2017). Late instar larvae can be distinguished from *P. charybdis* and the other well-established Australian paropsine beetle *Trachymela sloanei* (Gordon 2010), by their yellow colouration, distinct black median stripe from the mesothorax to abdominal segment VI, and black lateral strips (De Little 1979) (Figure 5.1g & h). Adults are obovate shaped, average 8 mm long (Cunningham and Murray 2007), with colour variations from pale brown, green, or yellow to orange (Figure 5.1i, j & k.). These colours fade away to dark brown after the beetles die. Elytral margins are widely expanded and translucent with waxy-look surface when alive. The underside of the beetles is distinctly black or dark in colour (Figure 5.1l).

*Pst. variicollis* feeds exclusively on *Eucalyptus* leaves and young tender shoots. The four larval instars of *Pst. variicollis* feed gregariously, while solitary adults make notches on the leaf margins. Larvae feed in groups, this gregarious behaviour and their aposematic colouring is thought to provide protection from predators. Aggregations of *Pst. variicollis* can be monospecific (with same or different instars) or heterospecific with other paropsine species such as *Paropsis atomaria* Olivier (Tan et al. 2017) and *Pst. agricola* (Chapuis) (De Little 1979). Like *P. charybdis*, *Pst. variicollis* rear up their abdominal apices in response to disturbance. *Pst. variicollis* also evert a pair of glands to release volatile toxic secretions (Moore 1967). Adult beetles usually drop to the ground in response to disturbance, or occasionally fly away.

Species formally in the genus *Chrysophtharta*, and now included in *Paropsisterna*, are typical cool-temperate forest and woodland species. Leaf beetle species under this genus from Australia prefer the temperate climate of south-eastern and south-western Australia (De Little 1979). In fact, *Pst. variicollis* has been recorded and is one of the predominant insect pests of economically importance in the cooler parts of Australia, i.e. Western Australia (Carnegie et al. 2005, Nahrung 2006), Southwestern Australia (Loch 2005), Victoria (Neumann 1993), the Australian Capital Territory (A.C.T.) and Tasmania (De Little 1979). *Paropsisterna variicollis* is recorded to have slightly different phenology in these different regions but generally it occurs on foliage from September to April. In four *E. globulus* plantations in Southwestern Australia, numbers of *Pst. variicollis* were recorded to increase in September and decreased during winter. Females were principally mature from September to January (Loch 2005, 2006). Adult beetles have been recorded to feed on tree tops from late November to April in Western Australia (Carnegie et al. 2005). However, in a *Eucalyptus gunnii* plantation in North Tasmania, they only occurred until January. *Paropsisterna variicollis* and *Pst. agricola* are thought to have different phenological adaptations to avoid direct competition with each other when on the same host trees (De Little 1979). *Paropsisterna agricola* appears early in the season while *Pst. variicollis* replaces it on the same or similar hosts trees in drier climates later in the season. In the Australian Capital Territory (ACT), *Pst. variicollis* has been noted as being common only

after January, later in the season than the more serious pest *P. atomaria* (Mo and Farrow 1993). Details of phenology were not recorded in these studies, but a bivoltine life cycle was observed in ACT (Carne 1966, cited in De Little 1979).


**Figure 5.1** a) and b) *Pst. variicollis* egg batches; c) and d) *P. charybdis* egg batches; e) *Pst. variicollis* 1st instars; f) *Pst. variicollis* mid instars; g) Aggregation of aposematic *Pst. variicollis* larve with mixed larval stages; h) Aggregation of aposematic *Pst. variicollis* late instar larvae with the same life stage larvae; i), j) and k) *Pst. variicollis* adults with various colours; l) Ventral view of *Pst. variicollis* adult showing distinct black colouration.

### 5.1.2. Chrysomelidae beetles in *Eucalyptus* plantation

Some of the paropsine chrysomelid beetles (Chrysomelidae: Chrysomelinae: Paropsini) are significant defoliators of eucalypts in Australia where eucalypt species are planted in commercial plantations outside their natural ranges, and around the world (Paine et al. 2011). These beetles generally share similar feeding patterns, consuming new shoots and young eucalypt leaves then move down to the lower part of the crown, creating a 'broom-top' crown (Loch 2005). Moderate or severe defoliation by the beetles can lead to reductions in tree growth (Elliott et al. 1993, Elek 1997, Quentin et al. 2010). In Australia, some native paropsine beetles became pests in eucalypt plantations where eucalypt species were planted outside their native ranges (Nahrung 2006). *Paropsisterna variicollis* is one of the paropsine pests species invading eucalypt plantations in Australia (Nahrung and Swain 2015). As a member of a complex of parosine species including *Pst. cloelia* (Stål) and *Pst. obovata* (Chapuis), all these pests of several *Eucalyptus* species, such as *E. camaldulensis*, *E. dunnii, E. globulus, E. grandis, E. gunnii, E. nitens* and *E. viminalis* (De Little 1979, Loch 2005, Nahrung 2006). In a survey in *E. globulus* plantations in Southwestern Australia, *Pst. variicollis* was the most abundant paropsine species, and was capable of developing 1-2 month outbreak populations (Loch 2005, 2006). It is also one of the predominant pests in Victoria for young eucalypts planted on farmland (Neumann 1993).

Due to the proximity to Australia, downwind position of the prevailing wind and frequent tourism and commercial trading, many Australian insects have established in New Zealand (Withers 2001). These include some paropsine beetles specializing on eucalypts, such as *Trachymela catenata*, *Ocrosopsis subfasciata* and well-established *P. charybdis* and *T. sloanei*, which have already caused significant defoliation in some New Zealand eucalypt plantations (Walsh 1998, Murphy and Kay 2000), and *T. sloanei* is also a pest in California (Paine et al. 2000). Several *Paropsisterna* spp. are known pests (Reid and De Little 2013), including three species in Tasmania where they are endemic (Elek and Patel 2016). The Tasmanian endemic *Pst. selmani* has caused significant defoliation of commercial *E. nitens* plantations in Tasmania since 1992 (Elek and Patel 2016), and has more recently become an invasive pest in Ireland (Horgan 2011). Another leaf beetle *Trachymela tincticollis*, first detected in 1982 in South Africa, can cause severe defoliation in a wide range of *Eucalyptus* species (Tribe and Cillie 1997). In Southern California, the combined effects of leaf beetles *Pst. m-fuscum* and *T. sloanei*, with psyllids can lead to significant damage to commercial *Eucalyptus* foliage production (Paine et al. 2011).

### 5.1.3. Objective

Because of the potential impact of *Pst. variicollis* on New Zealand eucalypt production and their wide host range in Australia, it is necessary to understand their host preference to assist existing eucalypt breeding programmes. This trial aimed to assess the initial incidence and defoliation caused by *Pst. variicollis* within durable eucalypt species trials already established close to the initial detection site. Controlled experiments could not be conducted as the beetle was, at the time, the focus of an open incursion response by the Ministry for Primary Industries, and as such no insect material could be handled or safely removed without compromising the response.

## 5.2. Methods

### 5.2.1. Study sites

Three durable *Eucalyptus* species trials in the Hawke's Bay region (Figure 5.2), (Alexander site 39° 37' 36.876"S, 176° 37' 45.4434"E, Hawke's Bay Regional Council site (HBRC) 39° 14' 54.2862"S, 176° 52' 15.3798"E, and McNeill site 39° 47' 21.0372"S, 176° 58' 13.3926"E), were visited from 18 to 26 January 2017 to assess incidence of and defoliation by *Pst. variicollis*. Average annual rainfall for each site is 797, 1484 and 1061 mm respectively. Trees were planted in 2011, 2013/14 and 2011/12 for the Alexander, HBRC and McNeill sites, respectively. Each trial consisted of multiples of single species plots of 49 trees at the Alexander and McNeill sites and 100 trees at the HBRC site, with plots of each species replicated and randomly arranged across each site. In total, 11 *Eucalyptus* species were assessed (Table 5.1). In each plot at the McNeill and Alexander sites, 30 inner trees (i.e. excluding perimeter trees) were assessed and 25 inner trees were assessed in the larger HBRC plots. Maximum tree top height was measured for all assessed trees using a 5 m E Reading staff (Accurate Instruments (NZ) LTD).

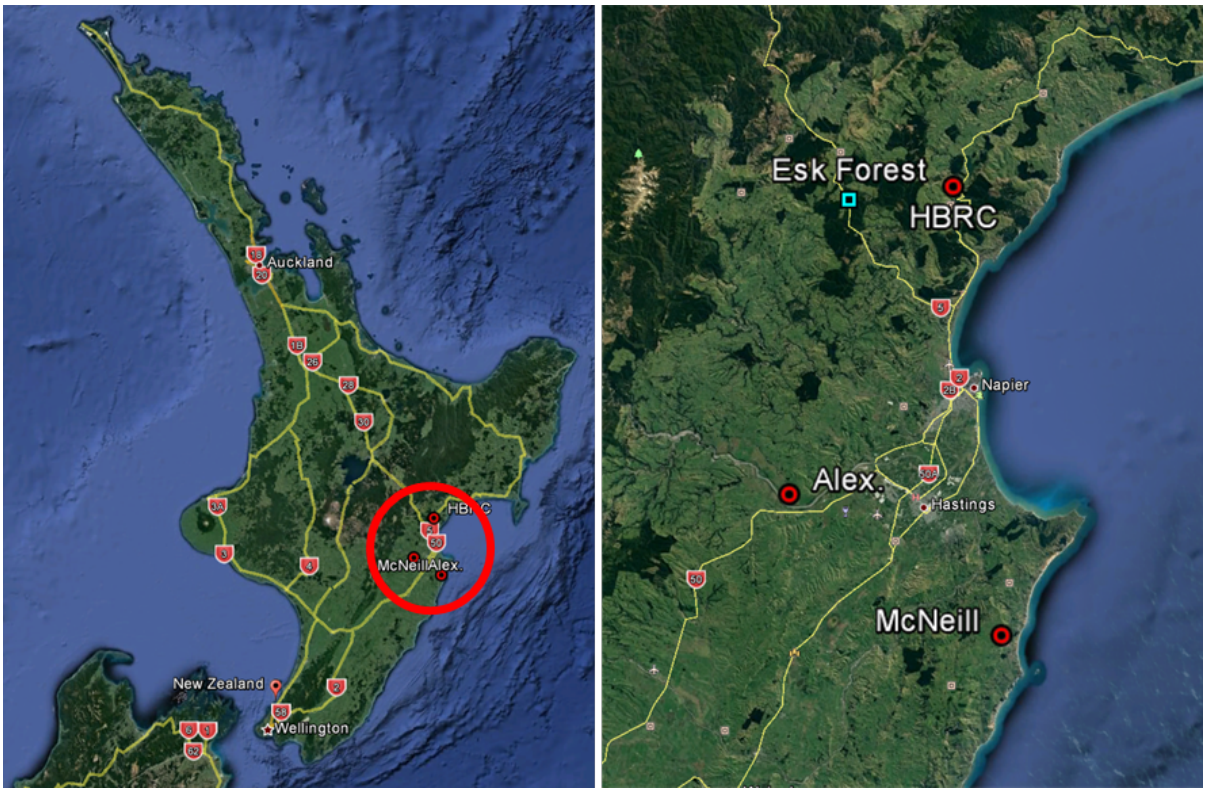



**Figure 5.2** Locations of the three study sites in the Hawke's Bay region of the North Island of New Zealand, and approximate location of original incursion site (a farm eucalypt shelterbelt running adjacent to Esk Forest) (Rogan 2016).

**Table 5.1** *Eucalyptus* species, number of plots sampled, and the total number of trees assessed at each of three Hawke's Bay sites for paropsine defoliation and, in parentheses, for eggs, larvae and adults of *Pst. variicollis* and *P. charybdis*. *Eucalyptus argophloia*, *E. eugenoides* and *E. notabilis* were absent (A) from some sites.

| Species | No. plots assessed | | | Total no. trees assessed | | |
|---|---|---|---|---|---|---|
| | Alex. | HBRC | McNeill | Alex. | HBRC | McNeill |
| *E. argophloia* | 2 | A | 2 | 38 (20) | A | 37 (26) |
| *E. bosistoana* | 4 | 2 | 2 | 92 (43) | 50 (30) | 46 (29) |
| *E. camaldulensis* | 4 | 2 | 3 | 94 (30) | 50 (25) | 70 (32) |
| *E. cladocalyx* | 2 | 2 | 2 | 35 (12) | 50 (31) | 46 (27) |
| *E. eugenoides* | 3 | A | 2 | 62 (23) | A | 69 (42) |
| *E. globoidea* | 3 | 2 | 2 | 61 (27) | 50 (27) | 46 (35) |
| *E. longifolia* | 3 | 2 | 2 | 45 (20) | 50 (23) | 50 (24) |
| *E. macrorhyncha* | 3 | 2 | 2 | 67 (25) | 45 (27) | 37 (24) |
| *E. notabilis* | 2 | A | A | 42 (20) | A | A |
| *E. quadrangulata* | 3 | 2 | 2 | 66 (22) | 50 (27) | 50 (37) |
| *E. tricarpa* | 3 | 2 | 3 | 72 (37) | 50 (26) | 71 (35) |

### 5.2.2. Experiment design

#### *Defoliation assessment*

Levels of defoliation caused by chewing by paropsine beetles were visually assessed for each tree in the selected plots and assigned a damage score: a = no or little chewing (<5%); b = light chewing (5–25% defoliation); c = moderate chewing (26–50% defoliation); d = moderately severe chewing (51–60% defoliation). No damage greater than 60% defoliation was observed. Grades were based on the incidence (proportion of leaves of the whole crown being damaged) and severity (proportion of damage per leaf) of damage (adapted from the CDI Crown Damage Index method of Stone et al. (2003), also in Chapter 4, section 4.3.2).

#### *Paropsine incidence*

Every second tree assessed for chewing damage was inspected closely for the presence of *Pst. variicollis* and *P. charybdis*. This resulted in the assessment of 46–102 trees per species, except *E. notabilis* (n=20 trees), which was present at only the Alexander site (Table 5.1). Three shoots that were reachable from the ground, and had similar amounts of foliage, were inspected per tree. Numbers of egg batches, larvae and adults of *Pst. variicollis* and *P. charybdis* were recorded. At the HBRC and McNeill sites, shoots were selected from different aspects and heights. However, at the Alexander site, trees were generally much taller, therefore shoots were selected from different aspects but only from the lower crown.

### 5.2.3. Statistical analysis

Defoliation data were analysed using the ordinal R package (Christensen 2015) in R (R Development Core Team 2008). Cumulative link mixed models were built with function clmm to analyse the effect of species on insect damage. Function lme4 (Bates et al. 2015) was used to build generalised linear mixed-effects models (GLMM) using function glmer to assess the impact of tree species on adult insect abundance only, as egg and larvae counts were too low for statistical rigour. Each explanatory variable was dropped in turn and the optimal model was selected using the likelihood ratio statistic and AIC. The R function for model selection was anova in base R.

## 5.3. Results and discussion

### 5.3.1. Defoliation assessment

Due to variable tree mortality (unrelated to this study) within plots at each site, the total number of trees available for assessment ranged from 135–219 per species, with the exception of *E. notabilis* (n=42) and *E. argophloia* (n=86), which were only present at one and two sites respectively. Most species at all three sites were assessed as suffering moderate to moderately severe paropsine chewing damage (Figure 5.3). Note that from a one-off visual assessment alone the causal agent (*Pst. variicollis*, *P. charybdis* or *T. sloanei*) of the leaf chewing could not be definitively known. Defoliation was highest at the McNeill site, with 26% and 71% of trees graded at damage levels c and d, respectively and no trees completely free of damage. The HBRC site, which was the wettest and had the youngest trees, also had the lowest damage levels (27% at levels a and b). However, there was no evidence that defoliation was correlated with average annual rainfall at a site.

Generally, *E. macrorhyncha* had the least chewing damage, followed by *E. cladocalyx* and then *E. globoidea*, the only monocalypt assessed. *Eucalyptus macrorhyncha* performed well at both the HBRC and Alexander sites, with 96% and 79% of trees respectively sustaining no or only light damage, but was more heavily defoliated at the McNeill site where > 50% of trees sustained moderate damage (Figure 5.3). *Eucalyptus cladocalyx* also suffered limited defoliation at both the HBRC and Alexander sites, (90% and 57% of trees showing only light damage), whereas all trees sustained moderate or moderately severe damage at the McNeill site. Performance of *E. globoidea* was more consistent, with mostly moderate damage at all sites (52%, 72% and 58% for the Alexander, HBRC and McNeill sites respectively).

More than 60% of *E. bosistoana*, *E. quadrangulata*, *E. camaldulensis*, and *E. argophloia* trees sustained moderately severe damage in all sites, with *E. bosistoana* and *E. quadrangulata* being the most badly damaged. More than 95% of *E. tricarpa* also suffered moderately severe damage at the Alexander and McNeill sites, but less than half suffered the same at the HBRC site. *Eucalyptus longifolia* varied consistently between moderate and moderately severe damage, and *E. notabilis* was badly damaged but only present at one site.

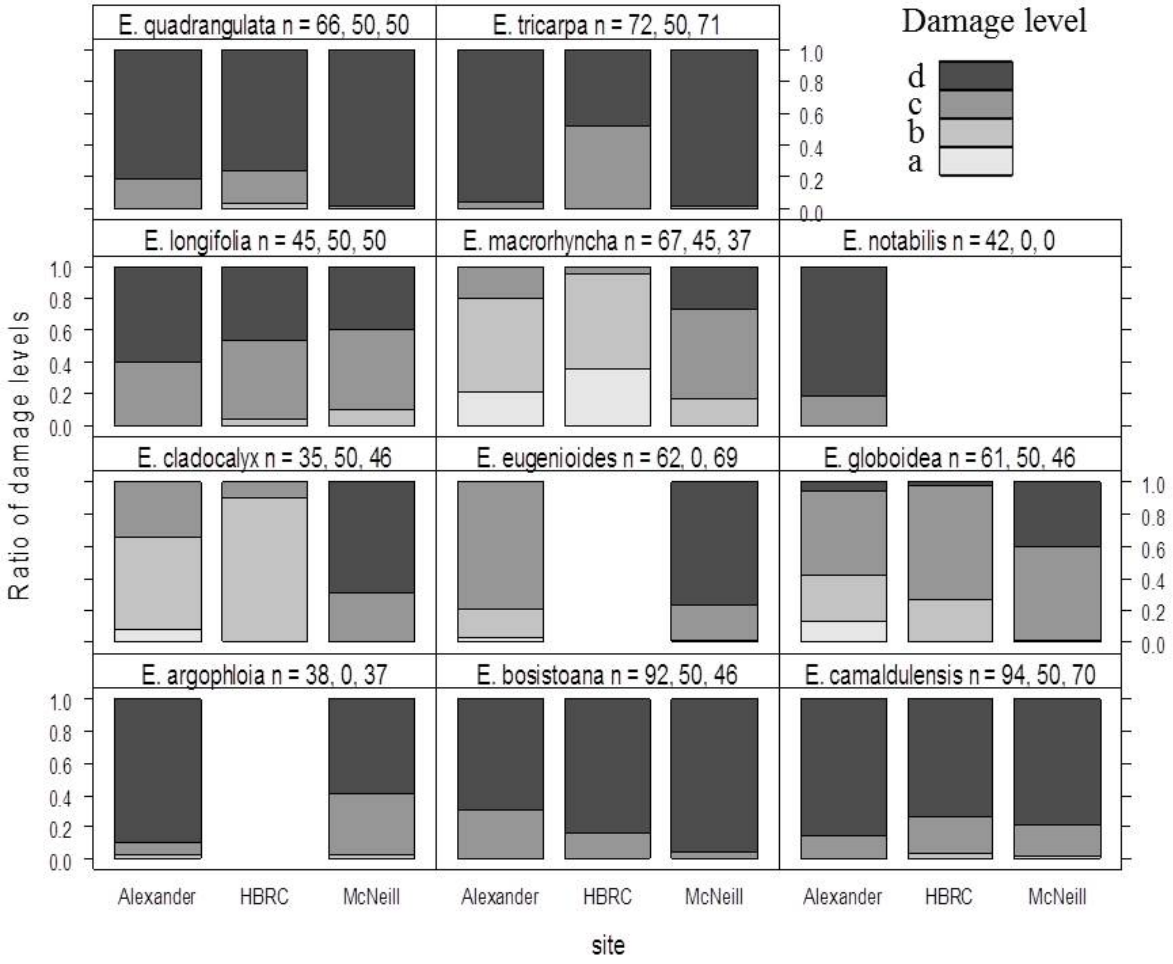



**Figure 5.3** Proportion of inspected trees assigned to each level of chewing damage (a = no or little chewing (<5%); b = light chewing (5–25% defoliation); c = moderate chewing (26-50% defoliation); d = moderately severe chewing (51-60% defoliation) for each *Eucalyptus* species across three study sites. The number of trees assessed at each site is indicated by n beside each species name respectively.

A cumulative link mixed model (CLMM) was fitted to analyse whether or not tree species and tree height affected defoliation. Only trees for which height data were available were used in the analysis. Fixed effects for the model were tree species and tree height (with interaction), and random effects were plot nested within site. Centring of tree height was achieved by subtracting mean tree height from each tree height value. Each explanatory variable was dropped in turn and compared with the full model to select the optimal model. Models without species as a factor (AIC=1806.5), without tree height (AIC=1747.9), and without interaction of tree species and height (AIC=1744.2), respectively, were significantly different from the full model (AIC=1721.7, $\alpha$=0.001). This result indicates that tree species and tree height significantly affected defoliation by paropsines. Predicted intercept estimates for each tree species at mean tree height (5 m) were produced for each damage level. Approximately 50% of *E. macrorhyncha* were predicted to incur no or light damage (Figure 5.4), but more than 80% of *E. tricarpa*, *E. bosistoana*, *E. quadrangulata*, *E. camaldulensis* and *E. argophloia* were

predicted to incur moderately severe damage, implying these species are more preferred hosts of *Pst. variicollis* and/or *P. charybdis*.

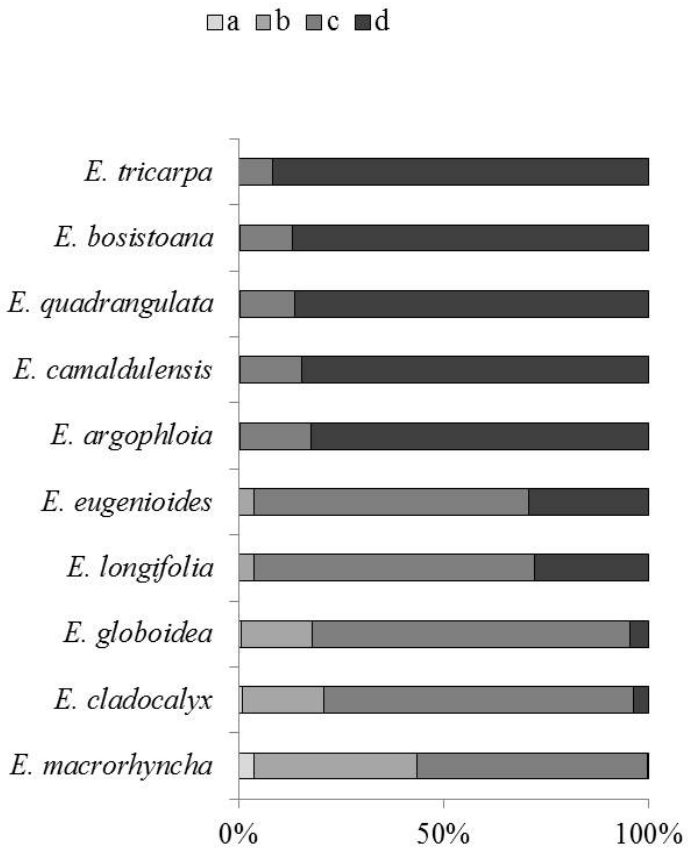



**Figure 5.4** Proportion of trees of each species predicted to appear in each insect damage category at the average tree height of 5 m. Values were extracted from CLMM model. *Eucalyptus notabilis* was only present in one of the three sites and is excluded as insufficient data were available for inclusion in this analysis.

### 5.3.2. Paropsine incidence

*Paropsisterna variicollis*, accounted for 73% of paropsine adults and nearly 100% of larvae and eggs, and was more abundant than *P. charybdis* at the Alexander and HBRC sites. Its abundance was similar to *P. charybdis* at the McNeill site. These results suggests that *Pst. variicollis* was the agent responsible for most of the chewing damage observed on average. The HBRC and McNeill sites had larger abundances of both species in all life stages than did the Alexander site. This may partly be due to the limitations of the sampling method which restricted sampling to shoots from the lower crown at the Alexander site, as the upper crowns were too high to reach.

The greatest abundance of *Pst. variicollis* adults were counted on *E. bosistoana* and *E. tricarpa* in all sites (Figure 5.5). In contrast, low counts were consistently found on *E. globoidea*, *E. longifolia*, and particularly *E. macrorhyncha*. *Paropsisterna variicollis* larvae counts were low on *E. cladocalyx* at both the Alexander and McNeill sites, but relatively high at the HBRC site

(about 1.3 larvae per shoot), where mean counts were skewed because 40 early instar larvae were found on a single shoot.

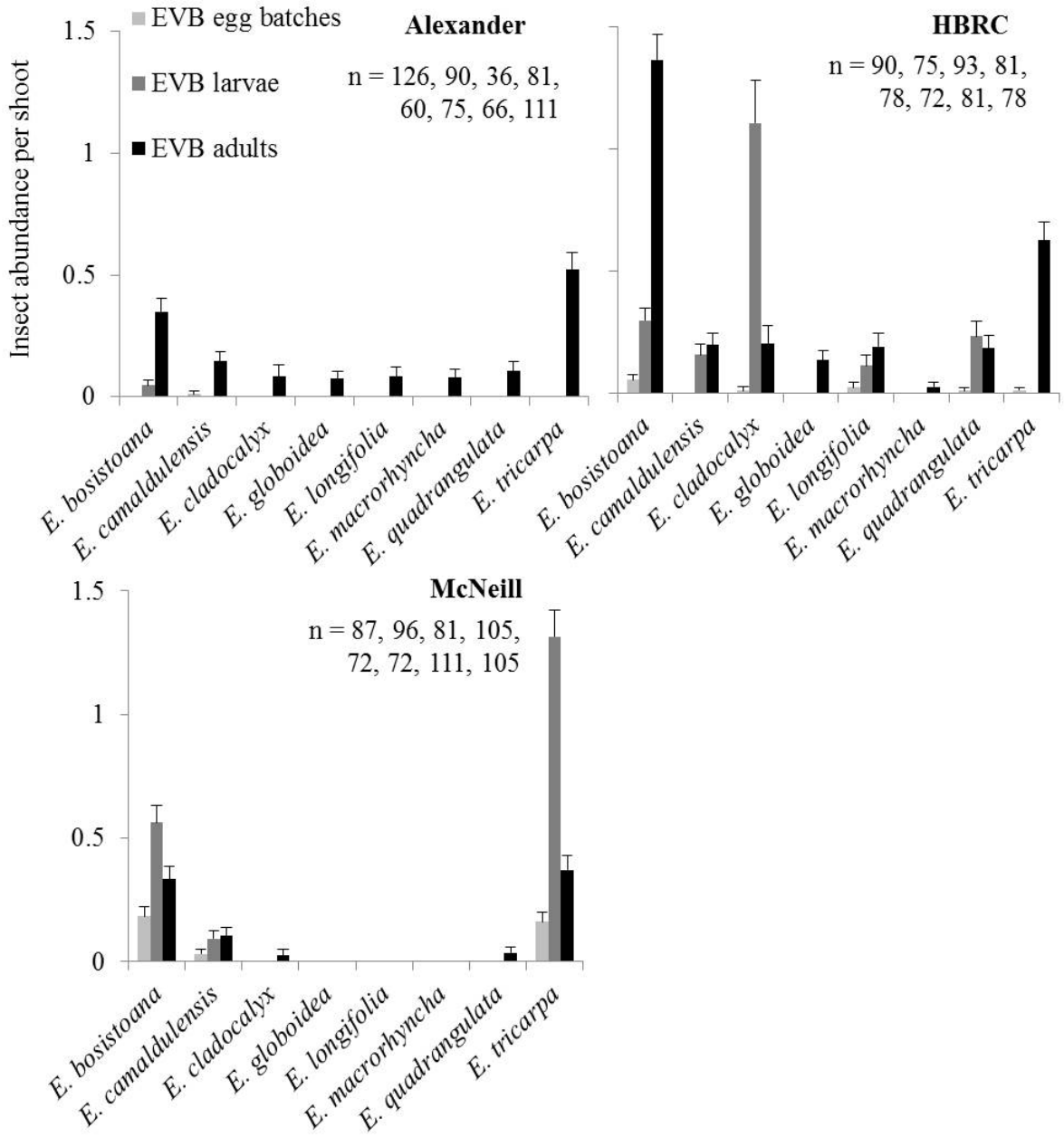



**Figure 5.5** Average (± SE) abundance of eggs, larvae and adult *Pst. variicollis* per shoot for the eight eucalypt species that were common to all three sites. The number of trees of each species inspected at each site is given as 'n' values representing each species in the order given on the x axis.

Counts of *Pst. variicollis* and *P. charybdis* eggs and larvae were near zero on most shoots sampled (Figure 5.5). This indicates the sampling period of 18 to 26 January used here may

not have been well timed to capture these important early developmental stages. Both Murphy and Kay (2000) and Jones and Withers (2003) noted January as being between the first (spring) and second (summer) generations of *P. charybdis* and this may have also been the case for *Pst. variicollis*. As such, a generalised linear mixed-effect model was fitted only to the count data for adult *Pst. variicollis*. A full model was built with tree species and height as fixed effects, and tree nested within plot nested within site as random effects. Interaction between tree species and height could not be examined due to a lack of data. Nested models without tree species (AIC=1888.453), tree height (AIC=1852.498) or both tree species and height (AIC=1890.222) respectively, were compared with the full model (AIC=1852.643) using the likelihood ratio statistic and AIC. The optimal model was that with only tree species as fixed effect, indicating tree species had a significant effect on *Pst. variicollis* adult abundance. Tukey-adjusted comparisons were used to compare post-hoc adult counts between each pair of tree species. *Paropsisterna variicollis* adults were significantly more prevalent on *E. bosistoana* and *E. tricarpa*, followed by *E. camaldulensis*, and significantly less prevalent on *E. macrorhyncha*. Differences among all other species were not significant.

### 5.3.3. Egg parasitism

Parasitism of *Pst. variicollis* eggs was detected on several occasions, in both HBRC and McNeill sites. As *Pst. variicollis* was still part of an active biosecurity response when conducting this trial, no eggs could be collected to confirm parasitism rates or parasitoid identity as either a new species, or as one of the three established species known to parasitise *P. charybdis* (*Enoggera nassaui* (Girault) and *Neopolycystus insectifurax* (Girault), (Murray et al. 2008)) or the Acacia beetle *Dicranosterna semipunctata* (unidentified Neopolycystus sp. (Murray and Withers 2010)). Late-stage parasitoid development was observed along with an adult parasitoid wasp assessing eggs, both of which were visually consistent with *N. insectifurax* (Figure 5.6a). Egg batches exhibiting colour patterns more consistent with parasitism by *En. nassaui* were also seen (Figure 5.6b). Both *En. nassaui* and *N. insectifurax* have been recorded from *Pst. variicollis* in the Australian Capital Territory (Mo and Farrow 1993) and *En. nassaui* has been recorded as parasitising *Pst. obovata*, which is thought to be part of a species complex with *Pst. variicollis* (T. Withers pers. comm.), in Tasmania (Murphy 2008). As such, parasitism of *Pst. variicollis* by *En. nassaui* and *N. insectifurax* would be expected in New Zealand. The species also has other natural enemies being recorded, such as tachinid flies as larval parasitoids. Loch (2006) indicates that *Pst. variicollis* appeared to be capable of developing short-lived outbreak populations but failed to create corresponding sharp increase in defoliation implying they may be controlled by natural enemies such as tachinid flies which can parasitize paropsine beetles larvae at high rates. Podapolipid mites (Acari:Podapolipidae) were found beneath the elytra of *Pst. variicollis* adults in Australia, but the impact on the beetles is unknown, although *Coccipolipus hippodamiae* (McDaniel and Morrill) infection on ladybirds can decrease their survival during overwintering and affect mating behaviours (Seeman and Nahrung 2005).

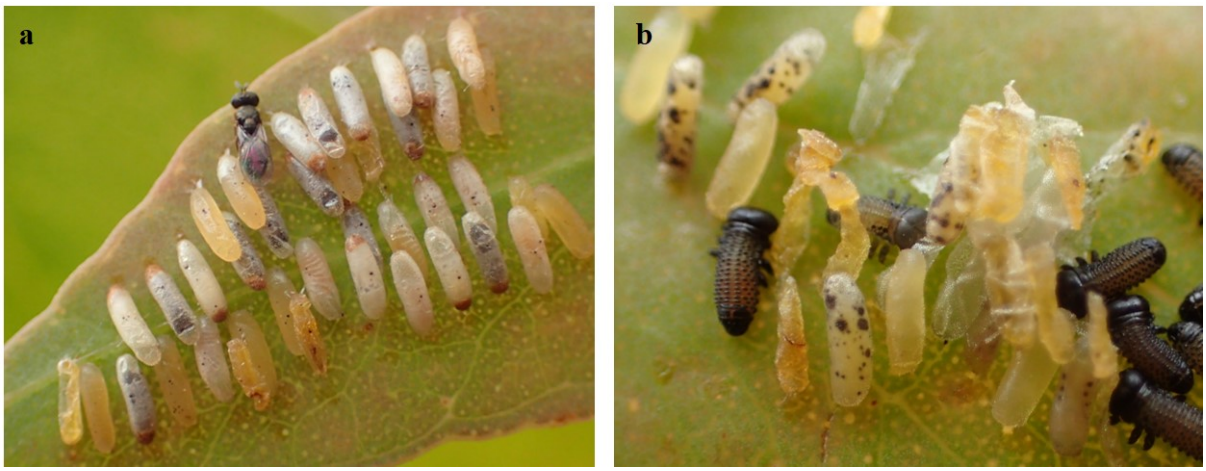



**Figure 5.6** Parasitised *P. variicollis* eggs observed at the McNeill site showing a) colour patterns similar to *N. insectifurax* parasitism of *P. charybdis* eggs and what appears to be an adult *N. insectifurax* assessing the eggs, b) colour pattern more consistent with parasitism by *Enoggera nassaui*.

## 5.4. Discussion and conclusions

Informal observations and formal delimitation surveys to date indicate *Pst. variicollis* is present and feeding voraciously on numerous eucalypt species within the Hawke's Bay region. This study attempted to quantify some of these observations in order to better understand the risk posed to a select group of eleven eucalypt species that were already established near the incursion point as part of an important new breeding programme for naturally durable timber. Adult beetles were observed on all 11 *Ecualyptus* species on three study sites. *E. bosistoana*, *E. quadrangulata* and *E. tricarpa* sustained the greatest defoliation, and *E. macrorhyncha*, *E. cladocalyx*, and *E. globoidea* the least. *Eucalyptus argophloia*, *E. camaldulensis*, *E. eugenoides*, *E. longifolia* and *E. notabilis* were intermediate. The greatest abundance of EVB eggs and larvae were on *E. bosistoana* while *E. macrorhyncha* and *E. globoidea* had the fewest. EVB was present in larger numbers than *P charybdis* in two of the three sites.

Shortly after the detection of *Pst. variicollis*, Rogan (2016) observed that, unlike the other established pest paropsines (*P. charybdis* and *T. sloanei*), larvae were still active in late April 2016. This raised concern within the wider eucalypt forestry industry that *Pst. variicollis* could become an even more damaging pest than these other species. Given previous experiences with *P. charybdis* and more recently the eradication attempt against *Paropsisterna beata* (Newman) (Yamoah et al. 2016), there is little reason to expect *Pst. variicollis* could not eventually spread throughout New Zealand wherever suitable host species are present if quick action is not taken to contain it. Exactly which eucalypt species will be suitable hosts, and the degree of impact that preferred species will sustain, is of intense interest as groups including the NZDFI are trying to expand the eucalypt industry and gain grower confidence. Results from two assessment methods used here indicate that, of the species assessed, *E. macrorhyncha* and *E. cladocalyx* are the least susceptible to *Pst. variicollis*, while *E. bosistoana*, *E. tricarpa*, *E. camaldulensis* and *E. quadrangulata* are the most.

The lack of *Pst. variicollis* eggs and larvae observed on most hosts, and the variability in damage levels sustained by each species between sites, may reflect the timing of the study, which possibly occurred between generations. A more reliable estimate of host preferences will

result from future studies that optimise sampling to detect oviposition and larval feeding. Variability in damage may also relate to the fact that defoliation could have been caused by a combination of *Pst. variicollis*, *P. charybdis* and *T. sloanei*. Although *Pst. variicollis* was detected with significantly higher abundance during this study, the other beetles, each of which may exhibit different species preferences, could have contributed to defoliation in the weeks prior to the assessment. The variability in defoliation is, however, also considered somewhat promising with regard to the NZDFI breeding programme. As numerous genetically distinct families are present in the trials (Apiolaza et al. 2011b), some of the variation in damage sustained could be linked to genetically heritable tolerance to defoliation and lower palatability, and this will be the focus of future assessments. Also encouraging was the observation that at least one species of parasitoid is successfully attacking *Pst. variicollis* eggs in the field which has potential to reduce population growth of the new pest beetle.

# CHAPTER 6 GENERAL DISCUSSION

This chapter will synthesise the findings from the experimental chapters 2-5, and highlight the implications for the sustainable integrated pest management of the developing New Zealand durable eucalypt industry and the wider plantation forestry industry. Major implications of the thesis cover the different but integral parts of IPM, including pest monitoring, defining control action thresholds and tree improvement (selective breeding) to reduce insect outbreaks. The findings from this thesis can be applied more broadly to pest management strategies for other forestry plantations and breeding programmes, especially those including exotic plantation species.

## 6.1. The need to manage pests in an integrated way

History shows there is no single effective pest management practise in agricultural and forestry systems that can fully mitigate insect pest impacts without harming the environment and/or human health. The harmful impacts of insecticides on the environment (e.g. soil and water) and biota (e.g. native and beneficial species) started to attract significant attention in the 1960s and 1970s (Liebhold 2012). Multiple sprays are often required to control pests, but these can be economically costly for farmers, and have issues like insecticide resistance of pests (Denholm and Rowland 1992) meaning that they are rarely effective in the long term. Although transgenic techniques (e.g. transgenic *Bacillus thuringiensis* (Bt) crops (Koziel et al. 1993)) have been suggested as a means to reduce the use of pesticides and lessen these environmental and pest management issues, evidence has shown the technology has its own downfalls such as transgenic contamination (e.g. gene flow from transgenic crops to wild relatives of crops (Chilcutt and Tabashnik 2004)), and losing effectiveness if insects evolve to resist the toxins (McGaughey et al. 1998). Like insecticides, transgenic methods are often not socially acceptable and are prohibited in plantations seeking FSC certification (Elek and Wardlaw 2013). In forestry plantations, especially monocultures, the benefits of selecting tree genotypes that are resistant to particular insects pests or pathogens, may not persist because the plantations are likely to provide enough food resources for insects to evolve and adapt to the few specific defensive traits (Henery 2011). Other pest management options, such as silvicultural practices that improve overall tree health, need to be used in conjunction with control measurements (e.g. attract-and-kill traps and biopesticides) to provide effective, sustainable pest management. Moreover, uncertainty around tree growth response to pest pressure due to changes in environmental conditions (e.g. climate change) and trophic interactions (e.g. new pest arrivals or changes in natural enemy abundance), require foresters to manage insect pests in a holistic and adaptive way.

## 6.2. Incorporating pest management in the initial stage of breeding programmes

This thesis aimed to answer questions concerning the risks and impacts associated with established and newly introduced insect defoliators on the developing dryland eucalypt industry in New Zealand. The findings, however, are broadly applicable to many types of plantation forestry. Chapters 2-5 resolved the objectives regarding three key components of IPM, which were pest monitoring, developing control action thresholds and selecting tolerant/resistant genotypes (species and families). Research outcomes of this thesis will facilitate an IPM strategy for eucalypt insect defoliators, and inform essential parts of the NZDFI breeding programme to reduce potential risk of insect outbreaks in the future. This will potentially increase the plantation productivity and minimise the use of insecticide for pest management (Figure 6.1). These IPM practices are vital for long-term plantation management, and will improve the competitiveness of the forest product by satisfying requirements necessary to gain access to restricted markets by holding Forest Stewardship Council (FSC) or other environmental certifications.

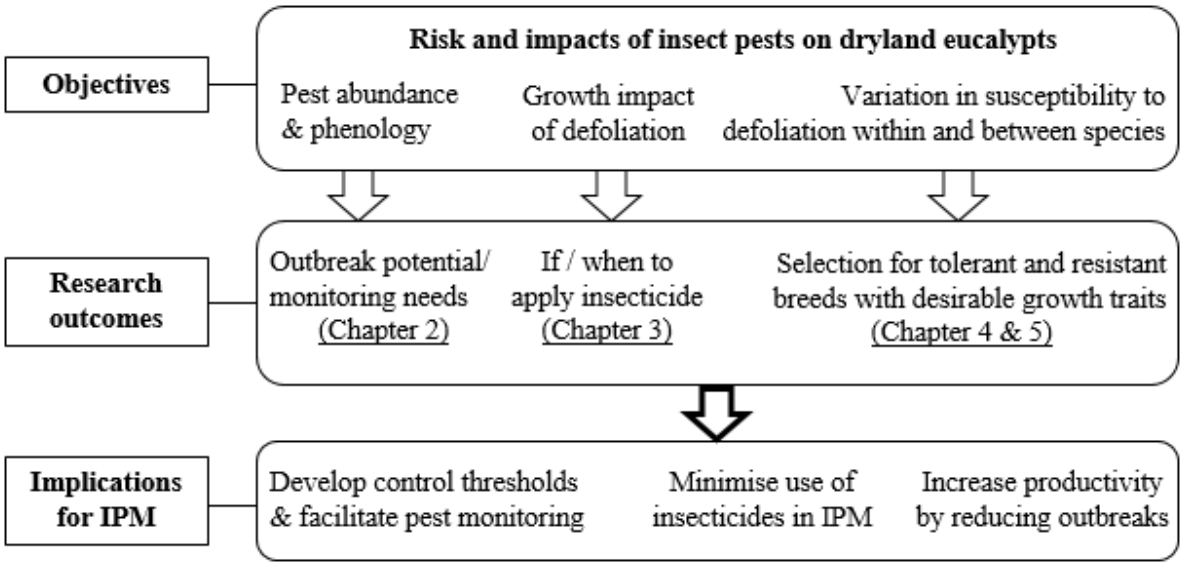



**Figure 6.1** Framework showing the links between thesis objectives, research outcomes and major implications for integrated pest management of eucalypt pests.

### 6.2.1. Implications for pest monitoring

The first two questions asked in this thesis( 1) what are the population dynamics of the four most common insect pests in a South Island dryland *E. bosistoana* plantation? and 2) for the most important pest, *P. charybdis*, can a simple degree-day model predict its voltinism and phenology?), examined the risk and monitoring needs of established insect defoliators. Differences in voltinism were identified in this study for *P. charybdis* and *O. eucalypti* compared to previous studies, indicating that site-specific pest management is essential to prevent unnecessary or ineffective use of pesticides in eucalypt plantations. *Paropsis charybdis* is the most important eucalypt pest of eucalypts grown for pulp in New Zealand, and was found to be the most damaging pest species of eucalypts for durable timber in the study site. Thus, *P. charybdis* requires the greatest monitoring effort. The simple degree-day model was able to predict generations of *P. charybdis* under some model scenarios (longer DD requirement (775.2°d) of median egg laying age and hibernation start by 20 March, or shorter DD requirement (623.2°d) of median egg laying age, hibernation start by 20 March and later overingwintering-adult emergence date (late September). Trapping will be needed to detect overwintering-adult emergence to determine the start date of the model, and hibernation start dates and duration will need to be investigated to improve the model performance in predicting voltinism of *P. charybdis*. Performance of the model in predicting the temporal appearance of life stages was not ideal, largely due to the lack of accurate data on the median egg laying age of adult beetles. If this information could be determined, the model could be adapted to assess the pest potential of *P. charybdis* in this and other sites by predicting the appearance of each life stage. This is a very important component of paropsine beetle IPM. In Tasmania, the abundance of leaf beetle eggs determines if additional monitoring and pesticide application is needed, as the abundance of eggs can be used to estimate future defoliation. If defoliation is predicted to exceed a certain threshold, insecticide is deemed necessary to control the insect population (Wardlaw et al. 2018). Ideally, control is conducted before the pests become late instar larvae as this stage causes the most damage (Edwards and Wightman 1984). Since *P.*

*charybdis* was observed to have overlapping life stages due to the long life span and egg laying period of adult beetles, control methods need to target not only the egg stage but also the larval stages to fully control the population. At present, the egg stage is partially controlled by *Enoggera nassaui* and *Neopolycystus insectifurax*, but insecticides are the only effective control option for larvae. However, the larval parasitoid *Eadya paropsidis* (Withers et al. 2017), which has been identified to attack the larvae stages of *P. charybdis*, is promising to improve the biological control on *P. charybdis* in concert with the two existing egg parasitoids.

Monitoring for the other three defoliators assessed was found to be less crucial than for *P. charybdis*, because populations were low and all caused only low levels of damage in the field. Monitoring of *O. eucalypti* may be needed in young plantations and nurseries, given young trees were observed to be completely defoliated by *O. eucalypti* in the field. Although foliage grew back the following season, this level of defoliation is likely to significantly reduce tree growth according to the findings in Chapter 3. Although *S. macropetana* did not cause significant damage, it was the most abundant pest species in the study. As damage mainly occurred to buds and new leaves, requiring energy to be put into the production of new leaves rather than stem growth, *S. macropetana* could potentially impact productivity and may therefore require monitoring in sites where it occurs in high abundance. As *Ph. froggatti*, occurred with low abundance, it is not considered to be a significant pest in dryland eucalypt plantations. Monitoring needs and outbreak potential of these three less significant defoliators may increase in more supressed plantations, due to their preference for slower growing trees.

### 6.2.2. Growth impact of defoliation: determining control action thresholds

Determining a control action threshold level of pest abundance, above which pesticide use is required to prevent economic loss, is one of the major components of constructing an IPM strategy. To determine this threshold, two steps are required: first, finding the lowest level of defoliation that can significantly reduce tree growth below an economically acceptable level and second, determining the pest abundance (observed through pest monitoring) corresponding to this defoliation level. In Chapter 3, it was shown that moderate (~50% of crown foliage) defoliation in spring did not significantly reduce tree growth, while moderate or severe defoliation in late summer did significantly reduce tree growth. Defoliation that occurred in both spring and late summer had greater negative impact on tree growth than single defoliation events. This indicates that insecticide control may not be necessary if trees are moderately defoliated in spring, but monitoring and modelling to predict if a second generation of *P. charybdis* will occur is needed to prevent damaging defoliation in late summer. However, although not statistically significant, growth gain of trees suffering moderate spring defoliation was lower than un-defoliated trees, which implies that continued monitoring of growth response may be required to understand the full effect of defoliation. Final productivity of trees sustaining moderate defoliation on an annual or less frequent basis needs to be compared to that of un-defoliated control trees at the end of a rotation to understand the defoliation impact in the long-term. Whether any reduction in productivity is acceptable will depend on the forest owners' needs and the trade-off between product volume and market access if allowing moderate defoliation means they can go pesticide free and get a higher level of environmental certification. The current study did not look at repeated defoliation over successive years but it is expected to have a cumulative effect on tree growth given spring plus late summer defoliation had a great impact on tree growth than spring or late summer defoliation alone.

### 6.2.3. Between and within species variation in insect susceptibility: implications for tree improvement

Chapters 4 and 5 addressed the objectives regarding variations in insect susceptibility within and between eucalypt species. The 11 durable eucalypt species as well as families of *E. bosistoana*, varied in pest load and insect damage. Within *E. bosistoana* variation in insect load and damage by *P. charybdis*, *S. macropetana* and *Ph. froggatti* were observed, which is consistent with previous studies showing variation in insect susceptibility in eucalpyts species and provenances of other eucalypt species (e.g. Mauchline et al. 2001, Farrell and Floyd 2007a). Similarly, in Chapter 5, differences in insect abundance and damage by *Pst. variicollis* (the newly established paropsine beetle) were significant between eucalypt species, and variation within species was also observed. Insect tolerance of the *E. bosistoana* families was ranked based on relative pest abundance and tree growth and damage sustained. For within-species variation in *E. bosistoana*, chewing defoliation (primarily caused by *P. charybdis*) of the crown was under 50% for all families. Families from the Southern provenance sustained the highest damage, generally between 10% and 30%, while trees within other families sustained less than 10% crown defoliation. Overall, although the Southern provenance families sustained the highest levels of chewing defoliation, they were also the tallest families relative to other families, suggesting they were more tolerant to damage despite high levels of pest attack. Southern provenance families were among the least susceptible to *S. macropetana* and *Ph. froggatti*. These results suggest that the Southern provenance families could be suitable for future breeding, because they were likely to be able to tolerate high levels of defoliation from *P. charybdis*, and were less susceptible by the insects that preferred suppressed hosts. Two families, *E. bosistoana* 125 and *E. globoidea* 999, had above average tree height and were less popular with all examined insects (except *O. eucalpyti* which showed no host preference between *E. bosistoana* families) compared to other assessed families. These families should be retained in the breeding programme. For *Pst. variicollis*, large variation in chewing damage was observed between sites and between and within eucalpyt species in three dryland eucalpyt plantations in the Hawke's Bay region. The most susceptible among the 11 eucalpyt species were *E. tricarpa* and *E. bosistoana*, while the least susceptible species were *E. macrorhyncha*.

The basis for selection for both pest tolerant and resistant species/genotypes lies in the complex variation in insect-plant interactions. This includes the effects of chemical and physical foliar properties, foliar nutrient availability, the phenology of both the host and pest, and variable growth response of different hosts to insect attack under a range of environmental conditions (Ohmart and Edwards 1991, Li 1994, Murray and Lin 2017). There is an enormous degree of variation (within and between species) in defensive chemistry and foliar phenology within *Eucalyptus* (Ohmart and Edwards 1991, Li 1994). The examined eucalypts species and families within *E. bosistoana* exhibited substantial variation in foliar (e.g. thickness and toughness of leaves) and crown morphology. Families within *E. bosistoana* showed variations in the temporal appearance of flush foliage and in leaf aging rate. These characteristics provide a potential basis for selecting suitable future breeds to reduce outbreak potential of *P. charybdis*, as the beetle prefers young soft leaves (Steinbauer et al. 1998, Steinbauer 2001), but more accurate measurements of leaf aging rates and the amounts of flush and expanding foliage should be used. Regardless of the species undergoing improvement as a plantation forestry crop, it is advantageous to screen out genotypes that are most susceptible to insect pests and

identify genotypes that are most tolerant to insect damage in the initial stage of breeding programmes. This selection can be integrated into existing breeding trials in which the priority is other commercial traits, such as fast growth rate and elite wood properties. However, if resistant genotypes (genotypes and suffer no insect attack) are detected in these programmes, they should also be maintained for further study, because these genotypes can provide an opportunity for studying key defensive traits of trees. Silvicultural practises such as planting trap trees (insect susceptible plants that are then sprayed), may also substantially delay resistance to defensive traits (Chilcutt and Tabashnik 2004). This approach, is not only applicable to dryland eucalypts plantations, but also to other forestry plantation breeding programmes.

### 6.2.4. Recommended approaches to dryland eucalypt IPM

This section summarises the recommendations on the aspects of specific IPM for durable eucalypts based on the results of the four experimental chapters.

#### *Recommendations on pest management of* P. charybdis

Comparing annual temperature and rainfall between the current study site and sites in previous studies (Clark 1930, McGregor 1989, Jones and Withers 2003, Nahrung 2006), suggests voltinism of *P. charybdis* may be significantly affected by rainfall. *Paropsis charybdis* tend to have more generations in moister sites relative to sites with drought conditions. This may be due to increased leaf toughness and a reduced ability to produce new leaves following defoliation (Larsson and Ohmart 1988) in drought conditions, factors which are critical to *P. charybdis* oviposition site selection and early larval development and survival. Therefore, moister sites are predicted to have higher risk of *P. charybdis* outbreaks, and thus, require more intensive pest monitoring. The site assessed in this thesis is one of the driest among the NZDFI trials, thus, more than one generation of *P. charybdis* could be expected to occur in some of the other NZDFI sites.

It is recommended monitoring of *P. charybdis* start from early-mid September in fortnightly intervals, given early instar larvae have been observed from early October. This will allow appearance and peaks of eggs and early instar larvae abundance to be detected. The frequency of early season monitoring could be reduced if the performance of the degree-day model were improved to more accurately predict when *P. charybids* beginning laying eggs of. This will require a more accurate estimate of adult emergence date to input into the model. To use egg or larval abundance to determine if control or further monitoring is needed the relationship between abundance and defoliation levels needs to be further investigated. The defoliation threshold is estimated to be ~50% defoliation of the crown, based on Chapter 3. If > 50% defoliation is predicted, control (e.g. insecticide or biopesticide) will be required to prevent growth loss. If 50% defoliation or less is predicted, no control is required, but monitoring needs to continue to detect if there is a second generation of *P. charybdis* (or using the degree-day model to predict if there is second generation), as repeated defoliation within a season can significantly reduce tree growth (Chapter 3).

Because *P. charybdis* exhibited overlapping life stages (Chapter 2), control needs to target both eggs and larvae to effectively suppress the population. Insecticide application needs to be

scheduled to maximise control of the stage it targets (e.g. larval stage of *P. charybdis*), but insecticides that can be used in combination with biocontrol that target different stages would be even better. Also, multi-species biocontrol programme (different biocontrol agents target different life stages of the pests) may be a better alternative to insecticide control if the control is adequate.

***Other recommendations on pest monitoring for dryland eucalypts***

Since *O. eucalypti* can cause severe defoliation of young trees, it may require monitoring in young plantations and nurseries. Egg abundance monitoring should be conducted in November and February to assess outbreak potential. Any eggs on small trees should be removed as even a small number of late instar larvae can cause severe defoliation on small trees. The arrival of *Pst. variicollis* during the course of this study highlights the fact that despite the biosecurity system in New Zealand being one of the strictest in the world (Eschen et al. 2015), ongoing annual surveillance is required in breeding trials due to the continuous risk of new insect incursions.

***Recommendations on methods of tree health assessment***

The different assessment methods used to rank family pest-susceptibility produced relatively consistent results. If the aim is to rank relative susceptibility of species or families the CDI (tree) method is the best, because although the other methods are more quantitative they are more time-consuming and take longer to analyse but produce much the same results. Susceptibility to *P. charybdis*, should be conducted from February, after insect abundance peaks, so that damage levels are stable. The same may be applicable to *Pst. varriicollis*, as its population dynamics and damage are similar to *P. charybdis*, but damage should be assessed after March as *Pst. variicollis* have been observed to remain active until April. If using the pest counting method (e.g. for *P. charybdis*), several assessments of egg and larval abundance need to be conducted to cover the full range of host preference, because host preference can vary throughout the season in response to variation in foliar phenology of hosts. For *S. macropetana*, susceptibility should be assessed during the hottest months to correspond with peak abundance.

***Recommendations on tree improvement for dryland eucalypts***

The significant variation in insect susceptibility between and within eucalypt species observed in Chapter 4 and 5, is promising for the NZDFI breeding trials because it indicates it could be possible to find genotypes with elite growth and wood properties that are also relatively pest tolerant or resistant. Insect susceptibility assessment can be extended to other existing breeding trials, which can potentially increase productivity and reduce impact on the environment.

*Eucalyptus bosistoana* families from the Southern provenance were more susceptible to *P. charybdis* but less susceptible to the other insects examined relative to Marulan and Bungonia provenance families. However, these families were taller than other families, which implied that they may have higher tolerance to insect defoliation. Insect tolerance of Southern provenance families should be further investigated in controlled experiments that also assess

diameter at breast height and stem volume. As *E. bosistoana* family 125 and *E. globoidea* family 999 had above average tree height a and were relatively less susceptible to all insect defoliators assessed, they are recommended for future breeding and further assessment with respect to insect resistance. The performance of *E. globoidea* 999 may imply that species within the subgenus *Monocalpytus* may be more suitable for future breeding when only considering tree growth and insect susceptibility, but as only one family was assessed further screening of more species and families is required. However, some *E. bosistoana* families performed as well as the *E. globoidea* family, and they are known to have greater durability of heartwood (Bush and Walker 2011), which indicates that the selecting of insect tolerant/resistant genotypes of eucalypts should not only consider the subgenus alone but should be also examined the species and family level. Similarly, *Pst. variicollis* was more abundant on and showed a preference for *Monocalpytus* species *E. tricarpa*, *E. quadrangulata* and *E. bosistoana*, and less abundant on *Symphyomyrtus* species *E. macrorhyncha* and *E. globoidea*, but there was significant variability between trees within each species tested.

## 6.3. Limitations and suggested future research

After determining the defoliation threshold, the next step of establishing control threshold of the IPM strategy in controlling *P. charybdis* is to quantify the relationship between the defoliation level and egg/larval abundance to reveal the egg/larval abundance that correspond to the defoliation threshold. This relationship has been determined for the IPM of leaf beetles in Tasmania (Elek and Wardlaw 2013), but as the beetle species, tree species and environmental conditions differ from those in New Zealand, the relationship is unlikely to be exactly the same for NZDFI *E. bosistoana* plantations (or other dryland eucalypt species) and needs to be quantified independently. In addition, the current study examined the impact of one or two defoliation events within a season, but did not examine the impact of chronic defoliation. Further study should explore the effect of chronic lower level defoliation (<50%) on durable eucalyt growth.

Due to time restrictions, pest phenology was only assessed at one site. Although this site represents a typical dryland eucalypt plantation pest phenology may vary due to micro-climatic conditions even in the same region. For example, *P. charybdis* was found to have different numbers of generations per year in two sites in south-eastern Queensland (Nahrung 2006). In the current study, peak egg abundance of *P. charybdis* was not observed which was likely due to monitoring was not started early enough in the season and low abundance of *P. charybdis* on the study site which may be attributed to the drought condition during experiment period. Assessing phenology at multiple sites will help determine how different climate factors interact to influence insect and plant phenology and how this influences pest abundance and impact on trees. Thus, further studies should be conducted in multiple sites to examine the insect phenology under different conditions. Also, the assessment interval needs to be reduced to more accurately detect population peaks and transfers between life stages. Data from such studies should be used to improve and validate the degree-day model developed here for *P. charybdis*. The same modelling approach could be applied to *S. macropetana* as a strong correlation was observed between larval abundance and temperature. Such studies can also help investigate to what degree *P. charybdis* voltinism is influenced by rainfall (or drought) in different sites, and its subsequent effect on tree growth and the physical and chemical properties of foliage.

To improve the performance of the degree-day model to predict the timing of *P. charybdis* life stages, the median egg laying age of *P. charybdis* adults (which was not examined in the current study due to a lack of available flush and expanding foliage) needs to be determined. A degree-day model was constructed for *Paropsisterna agricola* (Chapuis) in Nahrung et al. (2004), which the predicted first appearances of different life stages were within 2 weeks of their recorded occurrences in the field. The methods used to assess adult development and oviposition of *Pst. agricola* could be used as a reference for the further study of *P. charybdis*. Induced factor(s) (e.g. photoperiod and temperature) and start time of hibernation of *P. charybdis* adults and what life stages overwinter needs to be determined to further improve model performance in predicting voltinism. Future modelling also requires a direct measure of soil temperature to attain the real temperature experienced by pupae underground, and should look at the site-specific relationship between soil and air temperature to find out if air temperature can be used to deduce the soil temperature so that the modelling tool can be easily applied by forest managers in future IPM programmes.

To fully understand the insect-plant interactions in forest plantations, both pest phenology and variation in pest susceptibility between and within eucalypt species needs to be assessed in multiple sites. In Chapter 5, variation in insect damage was observed not only between sites, but also between eucalypt species in different sites. Assessing insect susceptibility at different sites will help understand the heritability of insect tolerance and resistance traits of eucalypt species and families. Also, control experiments that include measurements of the physiological response of trees to pest attack will help explain why some families have higher/lower tolerance to insect defoliation.

Foliar nutrients and chemical and physical properties of eucalypt foliage related to insect defence and tree vigour were not measured in the current study. As two out of 15 families (family 125 and 999) were relatively insect resistant and also fast growing, more families with these traits can be possibly found if screening is conducted at a larger scale. Examination of leaf toughness, leaf aging rate and defensive chemistry could help understand the mechanism of insect resistance/tolerance, and provide other clues to identify insect resistant or tolerant genotypes.

Besides the complexity of insect-plant interaction in forest plantations, influences of environmental factors, especially environmental stress, which could change such interactions, further highlight the need for IPM strategies in forest management. It has long been recognised by entomologists that environmental stress, such as drought, often precedes insect outbreaks, as a result of increased availability of leaf nutrients and/or reduced chemical defence (Koricheva et al. 1998). This hypothesis, which is called “Plant Stress Hypothesis”, has an opposite hypothesis called “Plant Vigour Hypothesis” (Whyte 2012), which argues that vigorous plants provide more and higher quality food resources and have less defensive compounds (Price 1991). Both hypothesises relate to the response of tree nutrient availability and chemical defence to the environmental stress, and the response of insects to changes in their hosts physiology. Evidence can be found supporting both hypothesises (e.g. White 1984, Inbar et al. 2001), and the debate is inconclusive. Conflicting results may be due to different host species responding in different way and the influences of the local environmental conditions. Also, changes in insect abundance during stressed conditions do not only result from the changes in the quality of their host plant, but are also influenced by changes in the abundance of natural enemies and the direct effects of the environmental factors themselves

(Koricheva et al. 1998). The dryland eucalypt trials may provide a good opportunity to examine these two hypothesises regarding the aspect of the impact of drought stress on insect-plant interactions.

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# APPENDICES

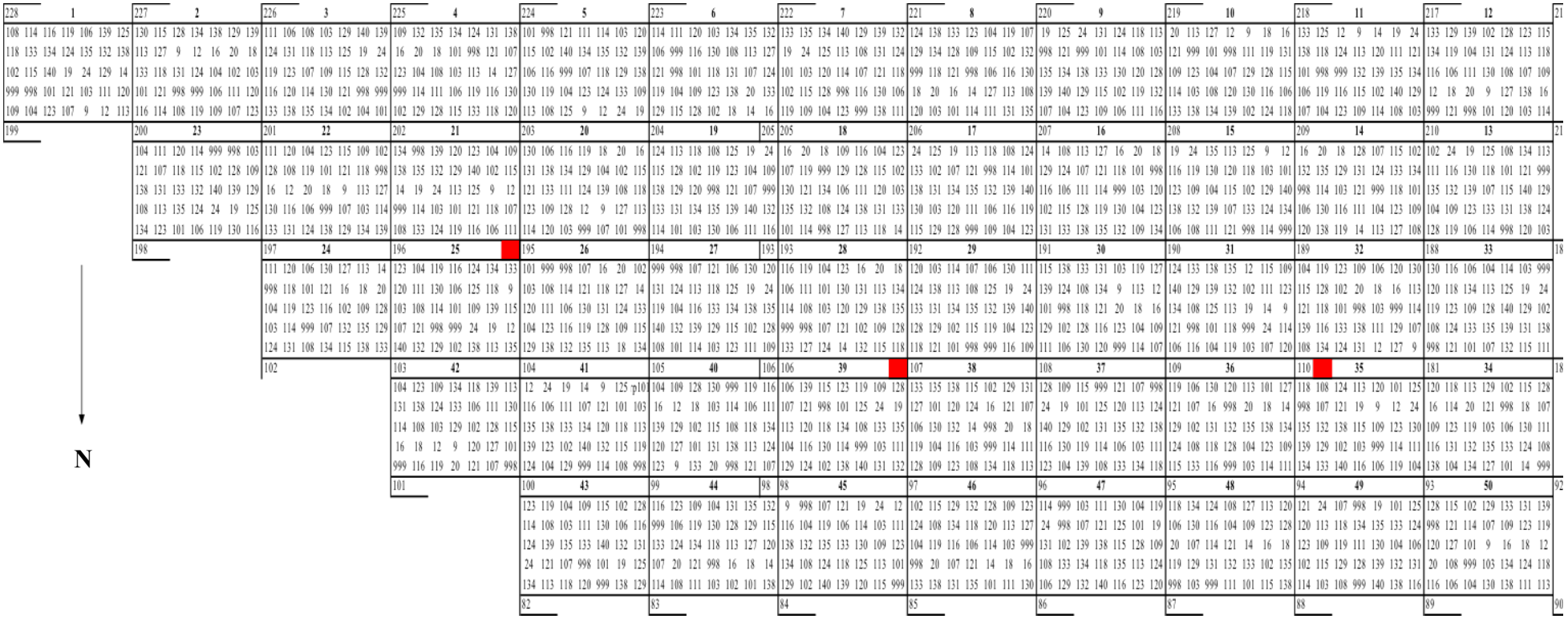
## Appendix 1: Summary of studies on defoliation impacts on the growth of *Eucalyptus*.

| Paper | Tree species | tree age; duration | Variables | Severity | Method and agent | Growth impact | Location |
|---|---|---|---|---|---|---|---|
| Lundquist and Purnell (1987) | *E. nitens* | 3 years; 2 year | provenances, severity | 0 - >75% | field; MLD | dbh was affected when defoliation level at 25-38%; height was affected when defoliation level at 38-50%. | South Africa |
| Candy et al. (1992) | *E. regnans* | 3 years; 4 year | severity, timing, frequency | 33%, 66%, 100% | field; artificial | Height, dbh significantly affected for all severity treatments. | Tasmania |
| | *E. regnans* | 6 years; 1 year | severity, timing, disbudding | 50%, 100%, 100%+disbudding | field; artificial | Height growth was significantly affected when defoliation level was 50% (disbud), 100% (disbud). | Tasmania |
| Abbott et al. (1993) | *E. marginata* | 1 year; 3 years | frequency, severity | 25%, 50%, 75%, 100% | field; artificial | 25%¬100% defoliation significantly affected dbh. | western Australia |
| Elliott et al. (1993) | *E. regnans* | 1 & 6years; 2 years | defoliation defoliation | natural defoliation | field; natural defoliation | Height increment significantly reduced. | Tasmania |
| Elek (1997) | *E. regnans* | 2-3 years; 7 years | defoliation | natural defoliation | field; natural defoliation | Defoliation had significant impact on dbh, height and volume. | Tasmania |
| | *E. nitens* | 3 years; 2 years | severity, disbudding | 50%, 100%, 100%+disbudding | field; artificial | Except 50%, defoliation significantly reduced dbh, height & volume. | Tasmania |
| Candy (2000b) | *E. nitens* | 2 years; 4-5 years | severity, timing, frequency, disbudding | 50%, 100% | field; artificial | Defoliation affected growth increment, late treatment produced a greater loss; repeat treatments (100%) had a greater impact than single year treatment. | Tasmania |
| | *E. nitens* | 2 years; 5 years | severity, disbudding | 50%, 100%, 100% disbudding | field; artificial | Defoliation had significant impact on growth. | Tasmania |
| Collett and Neumann (2002) | *E. globulus* | 1.7 years; 1.3 years | defoliation pattern, severity, repeated defoliation | 60% of lower/upper crown, 100% of lower/upper crown, 100% of total crown | field; artificial | Height increment reduced significantly after 100% of total crown and after two defoliation by 100% upper crown, but increased after 100% lower crown defoliation; Stem diameter affected by 100% of total crown defoliation. | Victoria |
| Floyd et al. (2002) | *E. globulus* | <1 year; 5 years | provenances | 0 - 85% (5% interval) | field; natural defoliation | Sprayed trees have greater volume than unsprayed trees. | south-east Australia |
| Jordan et al. (2002) | *E. globulus* | 4 years; 4 years | incident, severity, family | no, mildly, & severe damage | field; natural defoliation (*Perga affinis* ssp. *insularis*) | Mild and severe damage resulted in 16 and 31% loss in basal area (significant). | Tasmania |

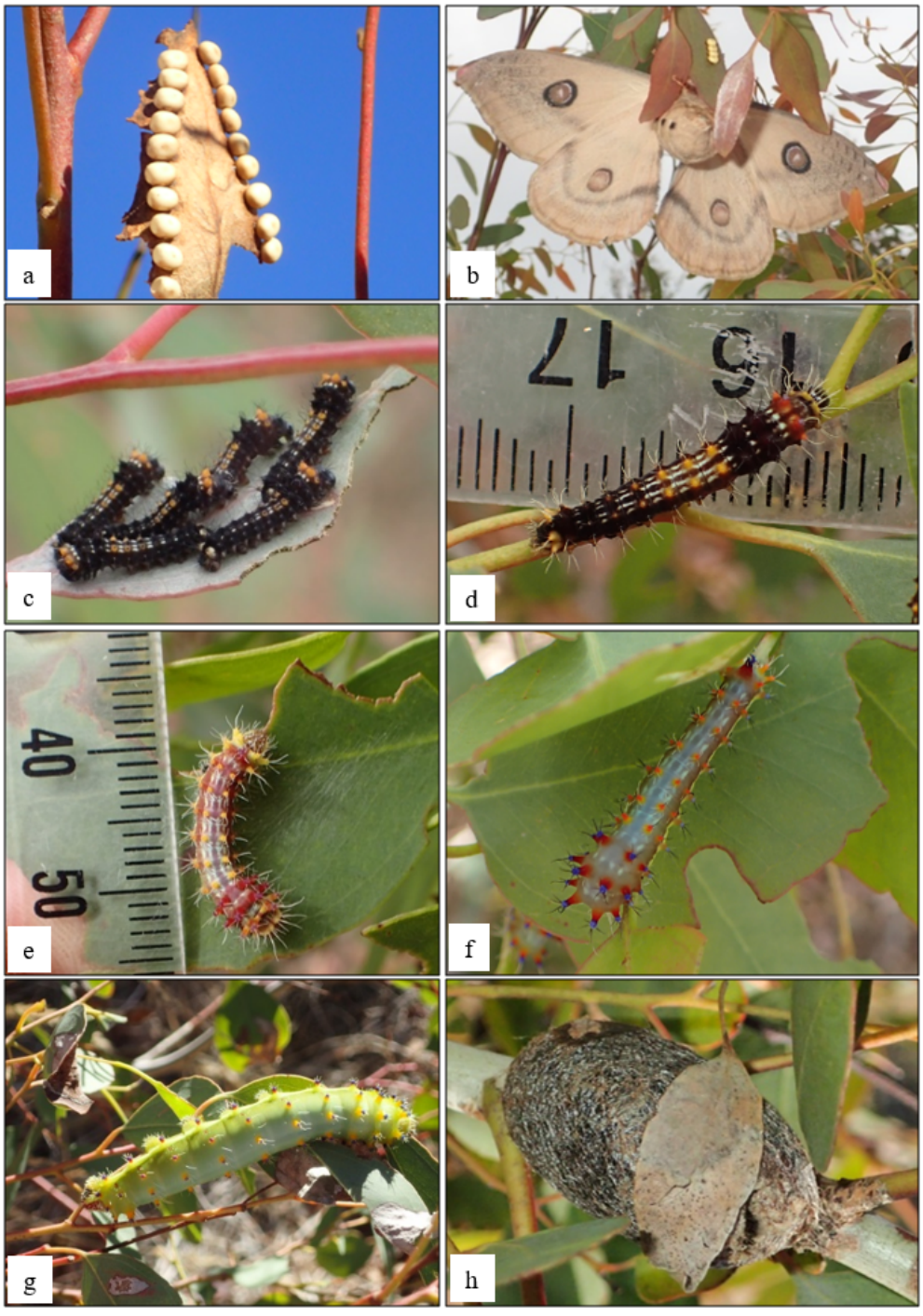
| Paper | Tree species | tree age; duration | Variables | Severity | Method and agent | Growth impact | Location |
|---|---|---|---|---|---|---|---|
| Carnegie and Ades (2003) | *E. globulus* | 2 years; 1.4 years | severity | 0 - 45% (10 scale) | field; MLD | <10% infection caused significantly poorer growth. | Victoria |
| Wardlaw (2004) | *E. globulus* | 1-2 years; 2 years | severity | natural defoliation (unknown severity) | field; MLB | Height and diameter growth were severely affected but recovered rapidly in the next growing season. | Tasmania |
| Wills et al. (2004) | *E. marginata* | 1 year/15 years | severity, frequency | 0, 100% | Field; artificial | Within 3 years, 100% defoliation significantly reduced diameter growth. | western Australia |
| Pinkard et al. (2006b) | *E. globulus* | 6 months /20 months | severity, defoliation pattern, frequency N | 25%, 38%, 50% | field; artificial | 25% throughout crown defoliation reduced dbh and height; 50% from upper crown reduced more dramatic than throughout crown on stem; more frequent, reduce more diameter. | Tasmania |
| Pinkard et al. (2006a) | *E. globulus* | 3 years; 1 year | N, P, defoliation | 0 - 50% | field; natural defoliation (*Gonipterus scutellatus*) | If no fertiliser, diameter and height increment were significantly reduced when defoliation was more than 10%. | Tasmania |
| Pinkard et al. (2007a) | *E. globulus* | <1 year; 1 year | severity, N, defoliation pattern, frequency | 25%, 38% | field; artificial | 25 and 38% defoliation significantly reduced stem diameter and height increment. | Tasmania |
| Farrell and Floyd (2007b) | *E. grandis* | <1 year; 6 years | provenance, severity | natural defoliation (0 - 100%) | field; natural defoliation | Insect damage significantly reduced plant volume over the experimental period. The genetically improved material had the greatest growth rate. | Murray Valley, Australia |
| (Farrell and Floyd 2007a) | *E. benthamii, E. dunnii, E. kartzoffiana, Corymbia* spps, *E. dorrigoensis,* | <1 year; 3 years | species, family, provenance, severity | natural defoliation (0 - 100%) | field; natural defoliation (*Mnesampela privata*, *Phylacteophaga* sppx) | Generally, no significant relationship between insect damage and seed lot growth but was significant for *E. dunnii* in one of the height class, though the relationships were not strong. | Murray Valley, Australia |
| Matsuki et al. (2007) | *E. globulus* | <1 year; 5 years | provenance, acute/chronic damage, | natural defoliation (0 - 100%) | field; natural defoliation (*Anoplognathus* spp. & other insect) | Severity and type of damage influenced growth rate, but the effect varied between years. | Murray Valley, Australia |
| Eyles et al. (2009) | *E. globulus* | <1 year; <1 year | nutrient, water, defoliation | 40% | field; artificial | No significant difference in stem and height growth between defoliation and defoliation treatments. | Tasmania |

| Paper | Tree species | tree age; duration | Variables | Severity | Method and agent | Growth impact | Location |
|---|---|---|---|---|---|---|---|
| Rapley et al. (2009) | *E. nitens* | 2 years; 6 years | severity | 1 - 100% (5 classes) | field, modelling; natural defoliation | Defoliation effect (on stand wood volumn) not significant until 60%, after that decline sharp. | southern Australia |
| Loch and Matsuki (2010) | *E. globulus* | 3.5 years; 3 years | severity, defoliation | 0 - 100% (>10 classes) | field; natural defoliation | Higher relative growth rates were recorded for insecticide treated trees in 2 of 4 plantations. | south-western Australia |
| Quentin et al. (2010) | *E. globulus* | <1 year; 1 month | defoliation methods | 25% | pot, glasshouse; artificial & managed insect defoliation (*Paropsisterna agricola*) | Artificial defoliation significantly affected diameter while real insect defoliation significantly affected both height and diameter. | Tasmania |
| Pinkard et al. (2011b) | *E. globulus* | <1 year; 1 year | water, N, severity | 50%, 75% of crown length (about 40%, 55% of leaf area) | field; artificial | Defoliation has no effect on growth increment in W-N-; increased increment in W-N+; reduced increment in W+N-, and in W+N+ after 2nd 75% defoliation. | Tasmania |
| Quentin et al. (2011) | *E. globulus* | 4 years; 1 year | defoliation | 50% of crown length (45% of leaf area) | field; artificial | Defoliation significantly reduced stem increment; Defoliation treatment had no effect on stem volume and biomass. | Tasmania |
| Barry et al. (2012) | *E. globulus* | 3 months; 1.3 year | defoliation, water, N | 1st, 50%of crown length; 2nd,70% of crown length | field; artificial | Defoliation has no significant effect on growth, but stem & bark volume and branch number were significantly increased in defoliated saplings. | Tasmania |
| Quentin et al. (2012) | *E. globulus* | 1 year; 0.5 year | water supply, defoliation | 75% of crown length | field; artificial | No significant effects of defoliation on growth. | Tasmania |
| Barry and Pinkard (2013) | *E. globulus* | 1 year; 4 months | defoliation, disbudding | 50% of crown length; 49% plus disbudding | pot, open growing area; artificial | Defoliation had significant effect on tree height and dbh over the experimental period, but increment and total leaf area were not significantly affected at the end of study. | Australia |
| | *E. nitens* | 1 year; 4 months | defoliation, disbudding | 50% of crown length; 44%(disbud) | pot, open growing area; artificial | | |
| De Oliveira et al. (2014) | *E. grandis* | 0.5 year; <1 years | severity | 50% & 75% of upper crown/ lower crown; 25% of the top; 100% | field; artificial | 100% defoliation cause reduction in diameter and height. | Brazil |
| Elek and Baker (2017) | *E. nitens* | 2 years; 13 years | Severity, timing, frequency, disbudding | 50% and 100% of adult leaves | field; artificial | Timing & frequency significant affected tree growth while severity & disbudding was not in the long term. | Tasmania |

**Appendix 2: The *E. bosistoana* study trial (in Chapter 2, 3, and 4) which is laid out in an incomplete block design: 48 of the 50 plots were used for the insect survey and family assessments, and each plot was made up of 35 trees (from different *E. bosistoana* families and 1 *E. globoidea* family) covering most of the 40 families represented at the site. Red squares represent the positions of three temperature loggers.**



**Appendix 3: The different life stages of *O. eucalypti*: a) egg batches; b) adult; c-d) early instar larvae; e-f) mid instar larvae; g) late instar larvae; g) pupa.**



**Appendix 4: Photos of *S. macropetana* a-b) larvae, and c-d) damage.**

**Appendix 5: Life stages of *Ph. froggatti*: a-b) mining damage; c) mining damage observed by shining a light through the leaf to observe the life stage; d) adult; e) cocoon in the mine; f) pupa.**

**Appendix 6: The different life stages of *P. charybdis*: a) egg batches; b) first instar larvae; c) 2nd, 3rd and 4th instar larvae (from right to left); d) pupa; e) pre-ovigenic adult; f) adults.**